



US 20100098627A1

(19) **United States**

(12) **Patent Application Publication**
Rohlf et al.

(10) **Pub. No.: US 2010/0098627 A1**

(43) **Pub. Date: Apr. 22, 2010**

(54) **PROTEINS**

(76) Inventors: **Christian Rohlf, Abingdon (GB);
Alasdair Stamps, Abingdon (GB)**

Correspondence Address:
**KLAUBER & JACKSON
411 HACKENSACK AVENUE
HACKENSACK, NJ 07601**

(21) Appl. No.: **12/547,743**

(22) Filed: **Aug. 26, 2009**

Related U.S. Application Data

(63) Continuation of application No. PCT/GB2008/
050124, filed on Feb. 25, 2008.

(60) Provisional application No. 60/903,509, filed on Feb.
26, 2007, provisional application No. 60/903,510,
filed on Feb. 26, 2007.

(30) **Foreign Application Priority Data**

Jun. 5, 2007 (EP) PCT/EP2008/055537

Publication Classification

(51) **Int. Cl.**
A61K 51/10 (2006.01)
A61K 51/08 (2006.01)
A61K 39/395 (2006.01)
A61K 38/16 (2006.01)
A61P 35/00 (2006.01)
G01N 33/68 (2006.01)
G01N 33/53 (2006.01)
G01N 33/48 (2006.01)

(52) **U.S. Cl.** **424/1.49**; 424/1.69; 424/133.1;
424/139.1; 514/12; 436/86; 436/501; 436/94

(57) **ABSTRACT**

The present invention provides methods and compositions for treatment, prevention, diagnosis and monitoring of colorectal cancer, kidney cancer and/or pancreatic cancer in particular relating to a polypeptide OGTA066.

OGTA066 (SEQ ID No: 1)

Peptide Source: 1D-GE, Colorectal cancer

MLHSALGLCLLLVTVSSNLAIAIKKEKRPPTLSRGWDDITWVQTYEGLFYAQSKKPLMVIHLE
DCQYSQALKKVFQNEEIQEMAQNKFIMLNLMHETTDKNLSPDGQYVPR**IMFVDPSTLTVR**ADIAGRYSN
RLTYEPRDLPLLIENMKALRLIQSEL

Mass Match Peptides (bold):

IMFVDPSTLTVR [3]

Tandem Peptides (underline):

IMFVDPSTLTVR [3]

Peptide Source: 1D-GE, Pancreatic cancer

MLHSALGLCLLLVTVSSNLAIAIKKEKRPPTLSRGWDDITWVQTYEGLFYAQSKK**KPLMVIHLE**
DCQYSQALKKVFQNEEIQEMAQNKFIMLNLMHETTDKNLSPDGQYVPR**IMFVDPSTLTVR**ADIAGRYSN
RLTYEPRDLPLLIENMKALRLIQSEL

Mass Match Peptides (bold):

IMFVDPSTLTVR [3]

KPLMVIHLEDCQYSQALK [4]

Tandem Peptides (underline):

IMFVDPSTLTVR [3]

Peptide Source: iTRAQ, Colorectal cancer

MLHSALGLCLLLVTVSSNLAIAIKKEKRPPTLSRGWDDITWVQTYEGLFYAQSKKPLMVIHLE
DCQYSQALKKVFQNEEIQEMAQNKFIMLNLMHETTDKNLSPDGQYVPR**IMFVDPSTLTVR**ADIAGRYSN
RLTYEPRDL**PLLIENMK**KALRLIQSEL

Mass Match Peptides (bold):

DLPLLIENMK [2]

IMFVDPSTLTVR [3]

Tandem Peptides (underline):

DLPLLIENMK [2]

IMFVDPSTLTVR [3]

Peptide Source: iTRAQ, Non-small cell lung cancer

MLHSALGLCLLLVTVSSNLAIAIKKEKRPPTLSRGWDDITWVQTYEGLFYAQSKKPLMVIHLE
DCQYSQALKKVFQNEEIQEMAQNKFIMLNLMHETTDK**NLSPDGQYVPRIMFVDPSTLTVR**ADIAGRYSN
RLTYEPRDL**PLLIENMK**KALRLIQSEL

Mass Match Peptides (bold):

DLPLLIENMK [2]

IMFVDPSTLTVR [3]

NLSPDGQYVPR [5]

FIGURE 1

OGTA066 (SEQ ID No: 1)

Peptide Source: 1D-GE, Colorectal cancer

MMLHSALGLCLLLVTVSSNLAIKKEKRPPQTLSRGWGDDITWVQTYEEGLFYAQKSKKPLMVIHLE
DCQYSQALKKVFQNEEQEMAQNKFIMLNLMHETTCKNLS PDGQYVPR IMFVDPSLTVR ADIAGRYSN
RLYTYEPRDPLLIENMKKALRLIQSEL

Mass Match Peptides (bold):

IMFVDPSLTVR [3]

Tandem Peptides (underline):

IMFVDPSLTVR [3]

Peptide Source: 1D-GE, Pancreatic cancer

MMLHSALGLCLLLVTVSSNLAIKKEKRPPQTLSRGWGDDITWVQTYEEGLFYAQKSK KPLMVIHLE
DCQYSQALK KVFQNEEQEMAQNKFIMLNLMHETTCKNLS PDGQYVPR IMFVDPSLTVR ADIAGRYSN
RLYTYEPRDPLLIENMKKALRLIQSEL

Mass Match Peptides (bold):

IMFVDPSLTVR [3]
KPLMVIHLEDCQYSQALK [4]

Tandem Peptides (underline):

IMFVDPSLTVR [3]

Peptide Source: iTRAQ, Colorectal cancer

MMLHSALGLCLLLVTVSSNLAIKKEKRPPQTLSRGWGDDITWVQTYEEGLFYAQKSKKPLMVIHLE
DCQYSQALKKVFQNEEQEMAQNKFIMLNLMHETTCKNLS PDGQYVPR IMFVDPSLTVR ADIAGRYSN
RLYTYEPR DLPLLIENMK KALRLIQSEL

Mass Match Peptides (bold):

DLPLLIENMK [2]
IMFVDPSLTVR [3]

Tandem Peptides (underline):

DLPLLIENMK [2]
IMFVDPSLTVR [3]

Peptide Source: iTRAQ, Non-small cell lung cancer

MMLHSALGLCLLLVTVSSNLAIKKEKRPPQTLSRGWGDDITWVQTYEEGLFYAQKSKKPLMVIHLE
DCQYSQALKKVFQNEEQEMAQNKFIMLNLMHETTCK NLSPDGQYVPR IMFVDPSLTVR ADIAGRYSN
RLYTYEPR DLPLLIENMK KALRLIQSEL

Mass Match Peptides (bold):

DLPLLIENMK [2]
IMFVDPSLTVR [3]
NLSPDGQYVPR [5]

Tandem Peptides (underline):

DLPLLIENMK [2]
IMFVDPSLTVR [3]
NLSPDGQYVPR [5]

Peptide Source: iTRAQ, Kidney cancer

MMLHSALGLCLLLVTVSSNLAIKKEKRPPQTLNRGWGDDITWVQTYEEGLFYAQKSKKPLMVIHHLE
DCQYSQALKKVFAQNEEIQEMAQNKFIMLNLMHETTDKNLSPDGQYVPRIMFVDPSLTVRADIAGRYSN
RLYTYEPRDLPLLIENMKKALRLIQSEL

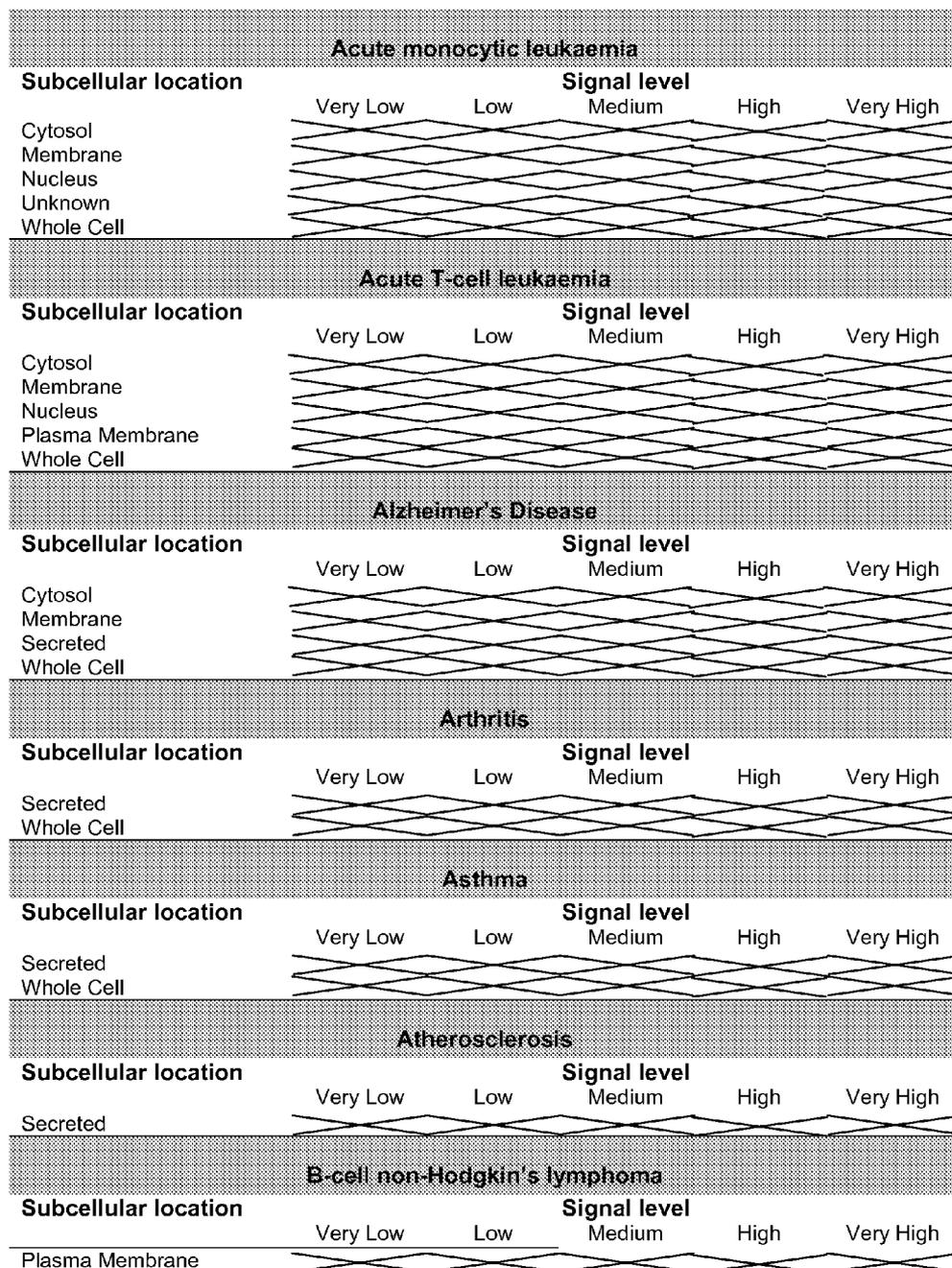
Mass Match Peptides (bold):

DLPLLIENMK [2]

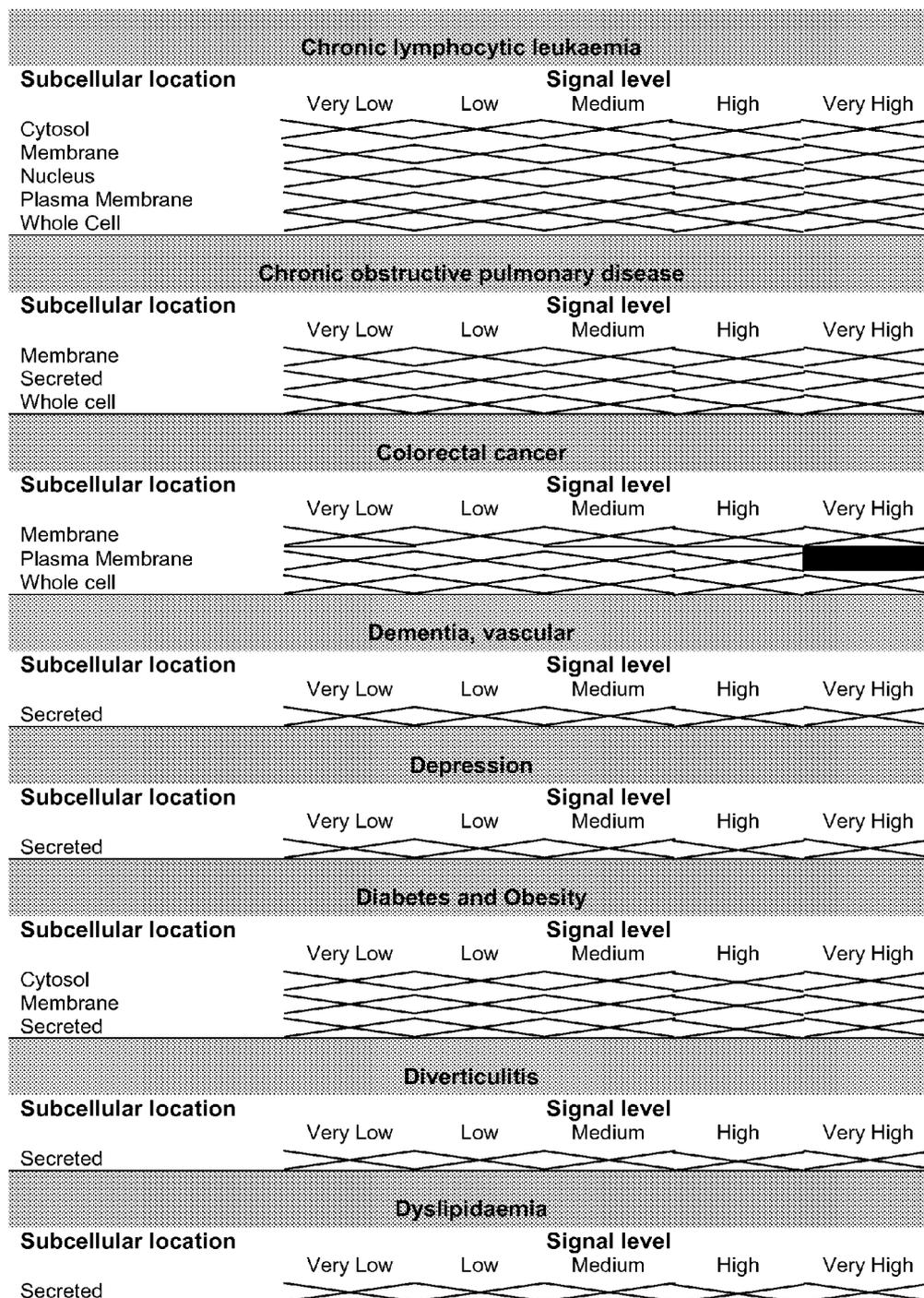
Tandem Peptides (underline):

DLPLLIENMK [2]

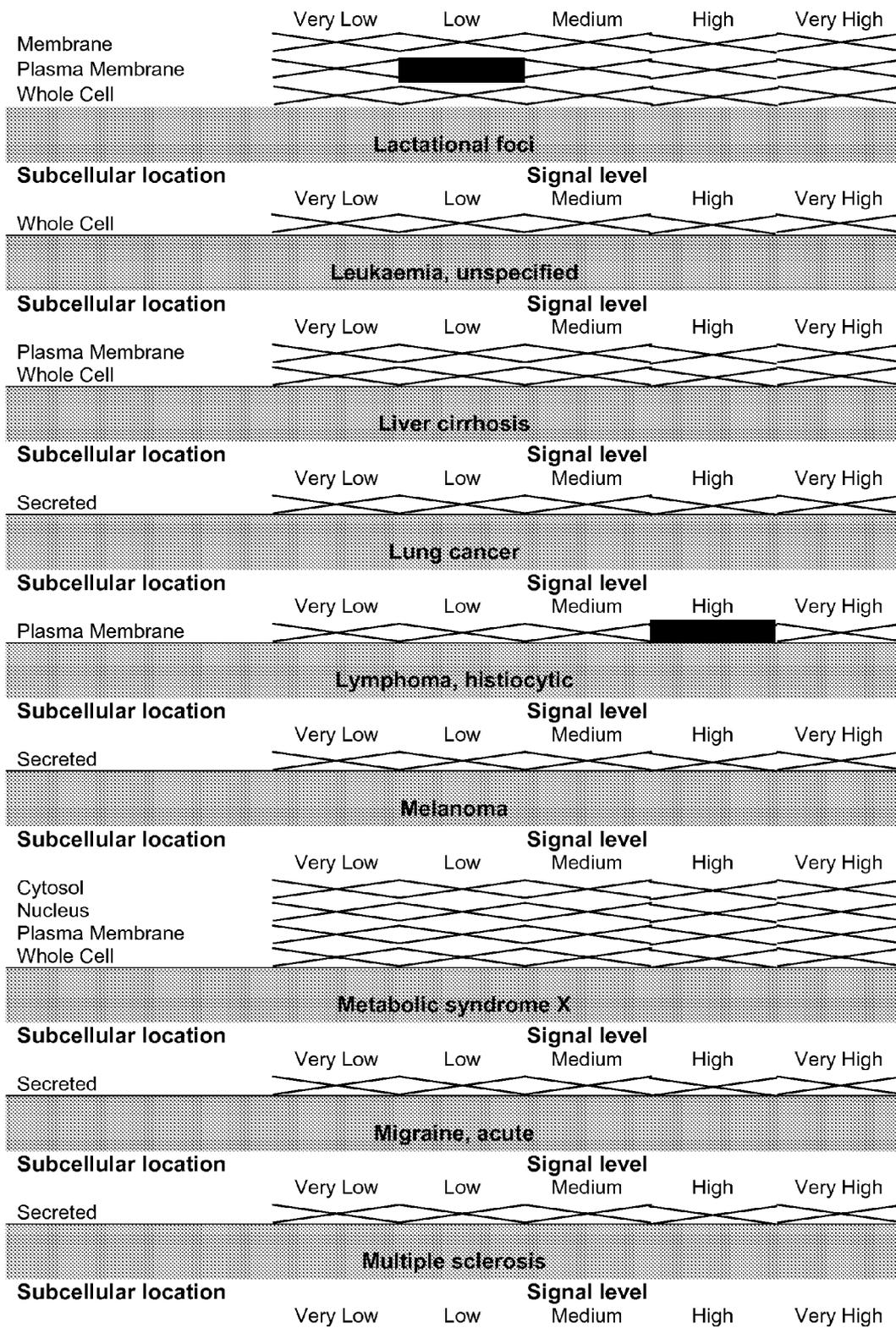
Figure 2

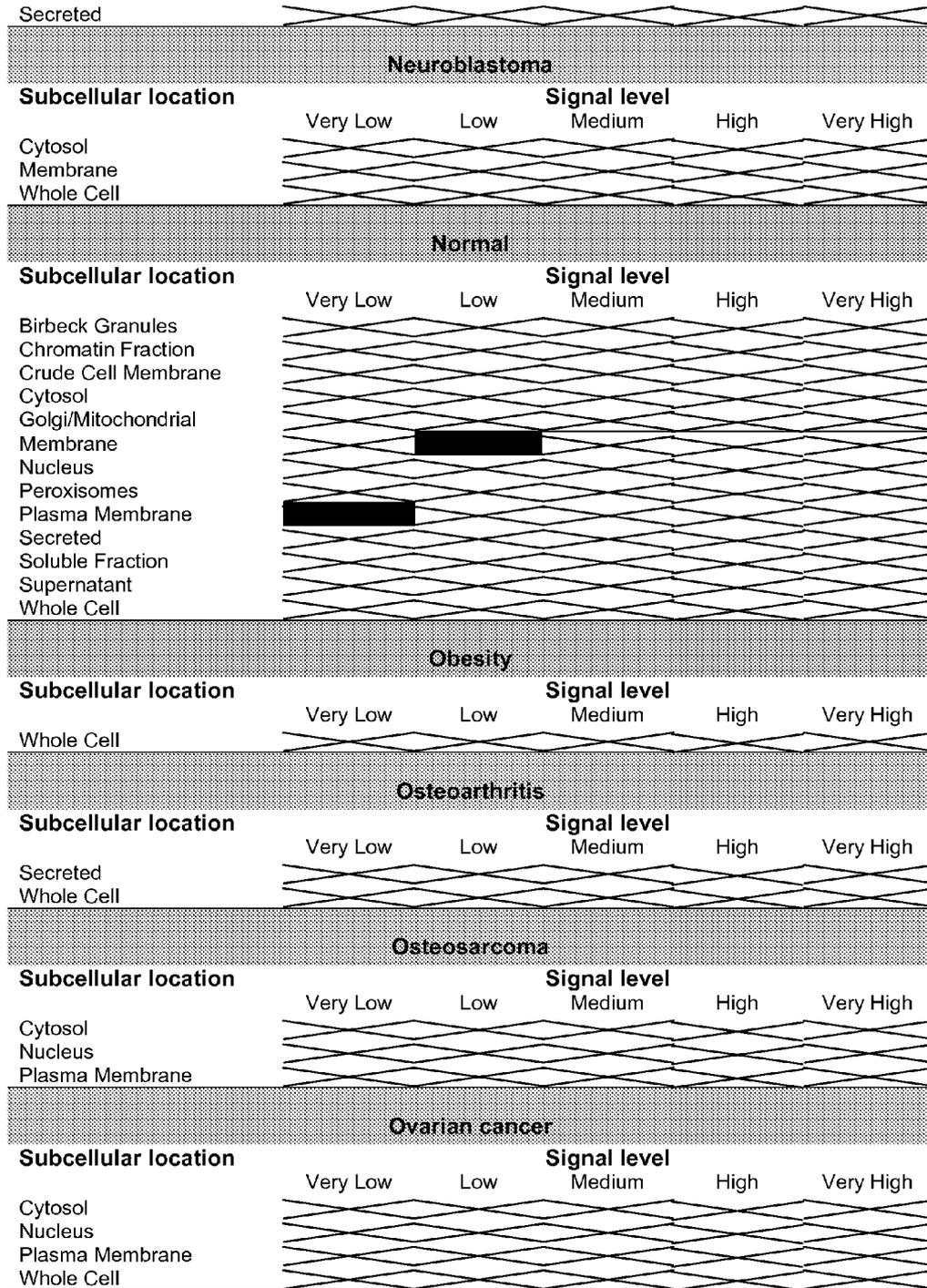


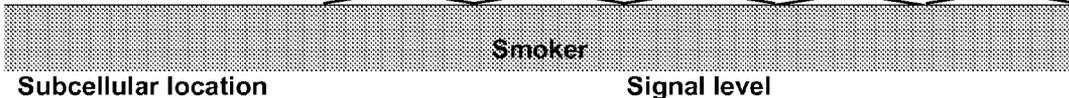
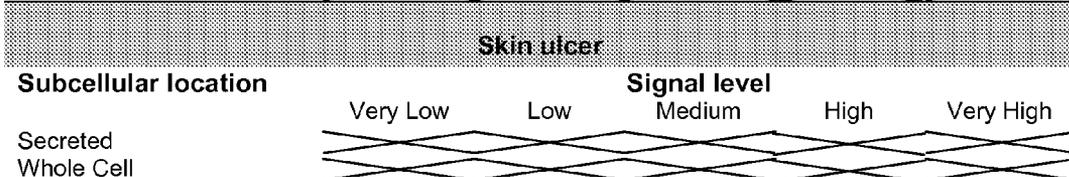
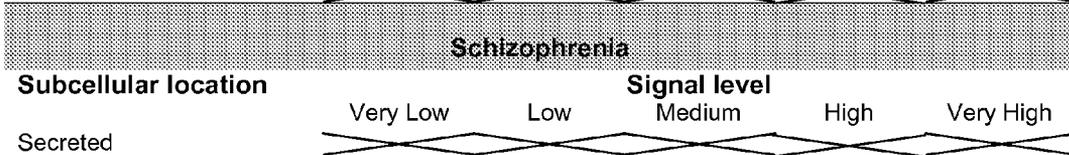
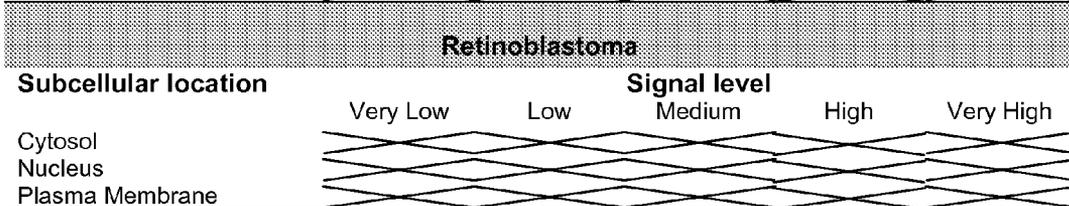
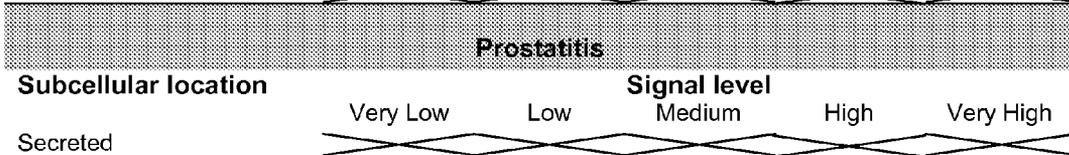
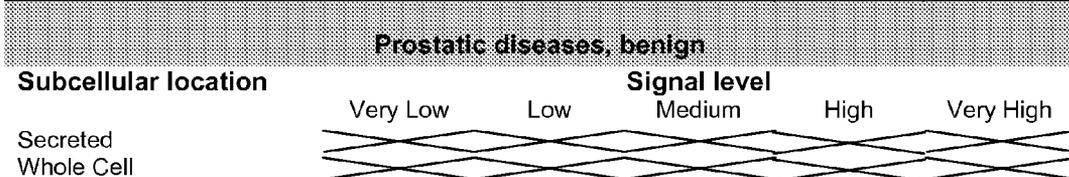
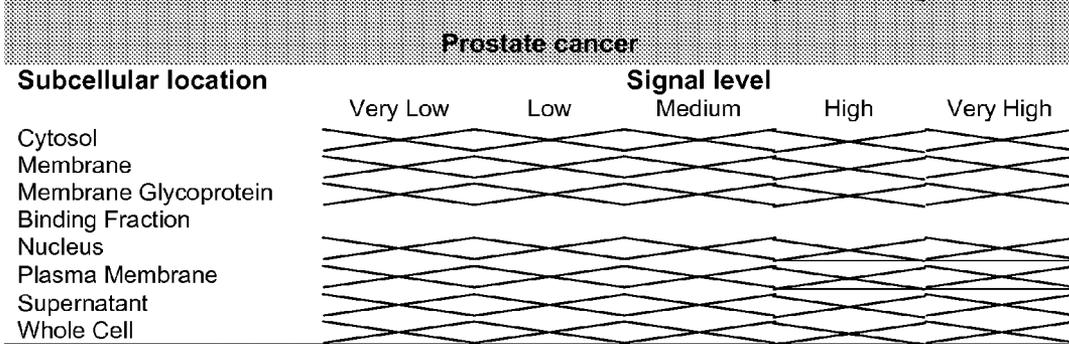
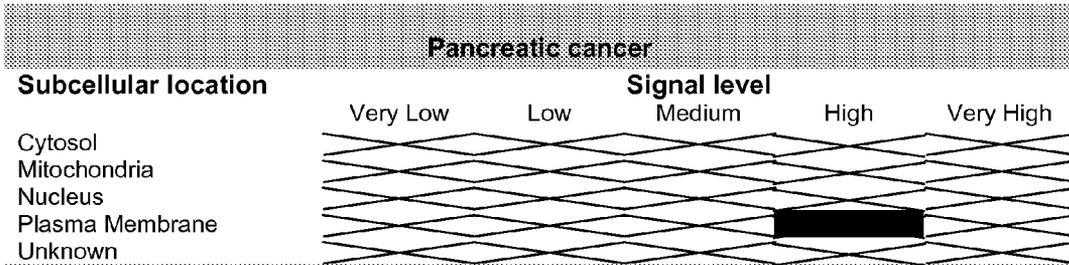
Bladder carcinoma					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Cytosol	[Cross-hatched]				
Membrane	[Cross-hatched]				
Nucleus	[Cross-hatched]				
Whole Cell	[Cross-hatched]				
Breast cancer					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Cytosol	[Cross-hatched]				
Membrane	[Cross-hatched]				
Mitochondria	[Cross-hatched]				
Nucleus	[Cross-hatched]				
Plasma Membrane	[Cross-hatched]				
Secreted	[Cross-hatched]				
Whole Cell	[Cross-hatched]				
Breast diseases, benign					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Secreted	[Cross-hatched]				
Burkitt's lymphoma					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Cytosol	[Cross-hatched]				
Membrane	[Cross-hatched]				
Nucleus	[Cross-hatched]				
Whole Cell	[Cross-hatched]				
Bursitis					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Secreted	[Cross-hatched]				
Cancer, unspecified					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Mitochondria	[Cross-hatched]				
Unknown	[Cross-hatched]				
Cervical cancer					
Subcellular location	Signal level				
	Very Low	Low	Medium	High	Very High
Cell surface digest	[Cross-hatched]				
Cytosol	[Cross-hatched]				
Plasma Membrane	[Cross-hatched]				
Whole Cell	[Cross-hatched]				



Emphysema					
Subcellular location	Signal level				
Whole Cell	Very Low	Low	Medium	High	Very High
Focal apocrine metaplasia					
Subcellular location	Signal level				
Whole Cell	Very Low	Low	Medium	High	Very High
Gastric cancer					
Subcellular location	Signal level				
Plasma Membrane	Very Low	Low	Medium	High	Very High
Gaucher disease					
Subcellular location	Signal level				
Secreted	Very Low	Low	Medium	High	Very High
Glioblastoma					
Subcellular location	Signal level				
Nucleus	Very Low	Low	Medium	High	Very High
Plasma Membrane	Very Low	Low	Medium	High	Very High
Whole Cell	Very Low	Low	Medium	High	Very High
Hepatoblastoma					
Subcellular location	Signal level				
Cytosol	Very Low	Low	Medium	High	Very High
Membrane	Very Low	Low	Medium	High	Very High
Nucleus	Very Low	Low	Medium	High	Very High
Whole Cell	Very Low	Low	Medium	High	Very High
Hepatocellular carcinoma					
Subcellular location	Signal level				
Membrane	Very Low	Low	Medium	High	Very High
Plasma Membrane	Very Low	Low	Medium	High	Very High
Secreted	Very Low	Low	Medium	High	Very High
Whole Cell	Very Low	Low	Medium	High	Very High
Hypertension					
Subcellular location	Signal level				
Secreted	Very Low	Low	Medium	High	Very High
Whole Cell	Very Low	Low	Medium	High	Very High
Kidney cancer					
Subcellular location	Signal level				







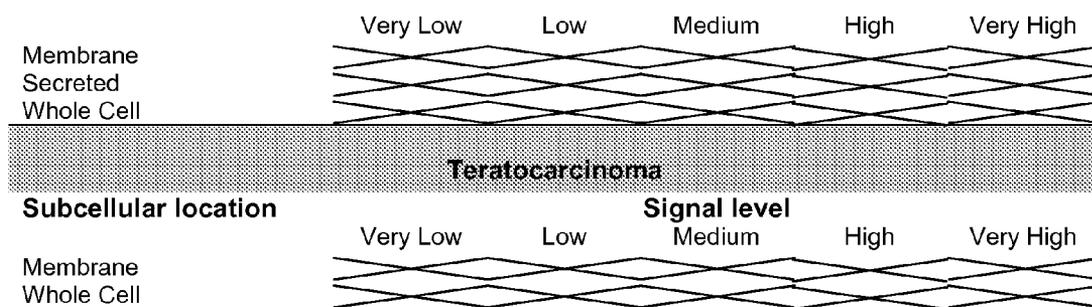
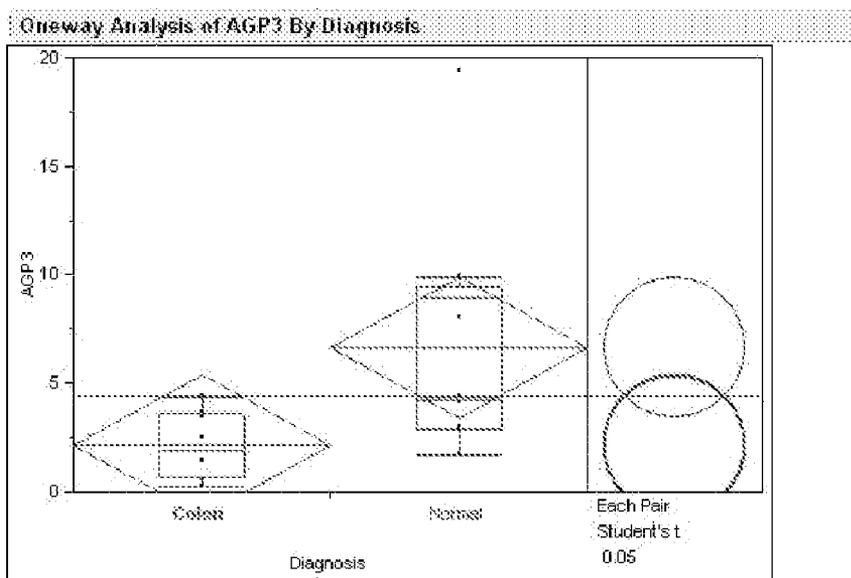


Figure 3

Box Plot data for OGTA066



Excluded Rows 16

Quantiles							
Level	Minimum	.10%	.25%	Median	.75%	.90%	Maximum
Colon	0.23	0.23	0.7	1.945	3.58	4.37	4.37
Normal	1.7	1.7	2.8525	4.205	9.435	19.37	19.37

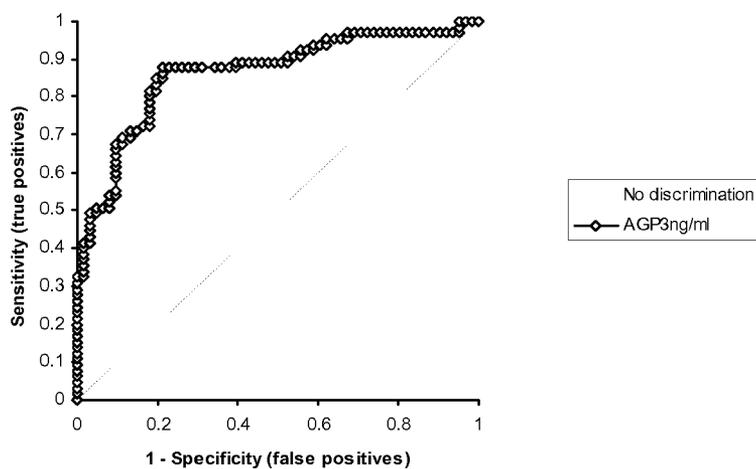
Oneway Anova					
Means for Oneway Anova					
Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Colon	8	2.16375	1.5110	-1.077	5.4044
Normal	8	6.64125	1.5110	3.401	9.8818

Std Error uses a pooled estimate of error variance

Means Comparisons					
Comparisons for each pair using Student's t					
Level	- Level	Difference	Lower CL	Upper CL	p-Value
Normal	Colon	4.477500	-0.105522	9.060522	0.0548

Figure 4

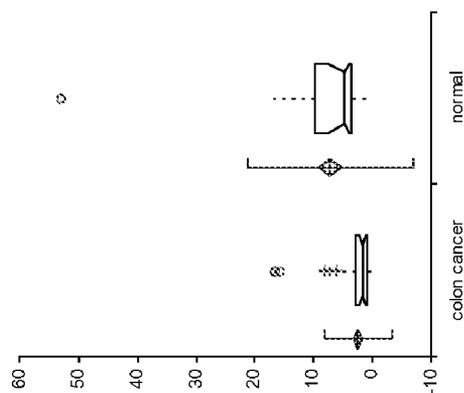
ROC curve data for OGTA066



Curve	Area	SE	p	95% CI of Area	Colon vs Normals = colon cancer
AGP3ng/ml	0.863	0.0334	<0.0001	0.797 to 0.928	have lower values

Figure 5

Box Plot data for OGTA066



AGP3ng/ml by Diagnosis	n	Mean	SD	SE	95% CI of Mean	Median	IQR	95% CI of Median
colon cancer	65	2.460	2.9292	0.3633	1.734 to 3.186	1.656	1.944	1.203 to 2.187
normal	61	7.248	7.1884	0.9204	5.407 to 9.089	4.849	6.050	3.985 to 7.451

PROTEINS

RELATED APPLICATIONS

[0001] The present application is a Continuation of co-pending PCT Application No. PCT/GB2008/050124 filed Feb. 25, 2008, which in turn, claims priority from PCT Application No. PCT/EP2008/055537 filed Jun. 5, 2007 and U.S. Provisional Application Ser. Nos. 60/903,509 and 60/903,510, both filed Feb. 26, 2007. Applicants claim the benefits of 35 U.S.C. §120 as to the first-referenced PCT application and priority under 35 U.S.C. §119 as to the second-referenced PCT application, and the said U.S. Provisional applications, and the entire disclosures of all applications are incorporated herein by reference in their entireties.

INTRODUCTION

[0002] The present invention relates to the identification of membrane protein associated with colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, and which may also form a biological target against which therapeutic antibodies (or other affinity reagents) or other pharmaceutical agents can be made, formulations/compositions comprising said protein/polypeptide, use of said polypeptide/protein or a composition comprising same in immunotherapy, antibodies for use in immunotherapy, compositions comprising a therapeutic antibody against a relevant polypeptide or a combination of antibodies and use of same in immunotherapy. The invention also extends to use of the relevant protein, fragments thereof or antibodies directed against the same for diagnosis of one or more of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer and kits comprising said protein, fragments or antibodies or fragments and use of said kits in methods of diagnosis.

BACKGROUND OF THE INVENTION

Colorectal Cancer

[0003] Colorectal cancer (CRC) is one of the leading causes of cancer-related morbidity and mortality, responsible for an estimated half a million deaths per year, mostly in Western, well developed countries. In these territories, CRC is the third most common malignancy (estimated number of new cases per annum in USA and EU is approximately 350,000 per year). Estimated healthcare costs related to treatment for colorectal cancer in the United States are more than \$8 billion.

Colorectal Cancer Diagnosis

[0004] Today, the fecal occult blood test and colonoscopy, a highly invasive procedure, are the most frequently used screening and diagnostic methods for colorectal cancer. Other diagnostic tools include Flexible Sigmoidoscopy (allowing the observation of only about half of the colon) and Double Contrast Barium Enema (DCBE, to obtain X-ray images).

Colorectal Cancer Staging

[0005] CRC has four distinct stages: patients with stage I disease have a five-year survival rate of >90%, while those

with metastatic stage IV disease have a <5% survival rate according to the US National Institutes of Health (NIH).

Colorectal Cancer Treatment

[0006] Once CRC has been diagnosed, the correct treatment needs to be selected. Surgery is usually the main treatment for rectal cancer, although radiation and chemotherapy will often be given before surgery. Possible side effects of surgery include bleeding from the surgery, deep vein thrombosis and damage to nearby organs during the operation.

[0007] Currently, 60 percent of colorectal cancer patients receive chemotherapy to treat their disease; however, this form of treatment only benefits a few percent of the population, while carrying with it high risks of toxicity, thus demonstrating a need to better define the patient selection criteria.

[0008] Colorectal cancer has a 30 to 40 percent recurrence rate within an average of 18 months after primary diagnosis. As with all cancers, the earlier it is detected the more likely it can be cured, especially as pathologists have recognised that the majority of CRC tumours develop in a series of well-defined stages from benign adenomas.

Colon Cancer Survival by Stage

[0009]

Stage	Survival Rate
I	93%
IIA	85%
IIB	72%
IIIA	83%
IIIB	64%
IIIC	44%
IV	8%

Kidney Cancer

[0010] Kidney cancer accounts for about 1.9% of cancer cases globally and 1.5% of deaths. Global incidence of kidney cancer is around 208,000 cases, with over 100,000 deaths. The incidence of kidney cancer is much higher in developed countries, being the sixth most common form of cancer in Western Europe. Around 38,900 new cases of kidney cancer are diagnosed in the USA each year, with around 12,800 deaths. It is very uncommon under age 45, and its incidence is highest between the ages of 55 and 84. The rate of people developing kidney cancer has been increasing at about 1.5% per year but the death rate has not been increasing. Renal cell carcinoma accounts for more than 90% of malignant kidney tumours. It has been estimated that approximately US \$1.9 billion is spent in the USA each year on treating kidney cancer.

Kidney Cancer Diagnosis

[0011] Many renal cell cancers are found at a late stage; they can become quite large without causing any pain or discomfort and there are no simple tests that can detect renal cell cancer early. About 25% of patients with renal cell carcinoma will already have metastatic spread of their cancer when they are diagnosed.

[0012] Renal cell cancer can often be diagnosed without the need for a biopsy using a CT scan, MRI, ultrasound, positron emission tomography (PET) scan, intravenous pyelogram (IVP) and/or angiography. Fine needle aspiration biopsy may however be valuable when imaging results are not conclusive enough to warrant removing a kidney.

Kidney Cancer Staging

[0013] Renal cell cancers are usually graded on a scale of 1-4. Renal cell cancer is also staged using the American Joint Committee on Cancer (AJCC) TNM system—stage I-IV. The University of California Los Angeles Integrated Staging System can also be used, which divides patients without any tumour spread into three groups—low risk, intermediate risk and high risk. The 5-year cancer-specific survival for the low-risk group is 91%, for the intermediate-risk group is 80%, and for the high-risk group is 55%. Patients with tumour spread are also divided into three groups—low, intermediate and high risk. The 5-year cancer-specific survival for the low-risk group is 32%, for the intermediate-risk group 20% and for the high-risk group 0%.

Kidney Cancer Treatment

[0014] Surgery by radical nephrectomy (and sometimes regional lymphadenectomy), partial nephrectomy or laparoscopic nephrectomy is the main treatment for renal cell carcinoma. Renal cell carcinomas are not very sensitive to radiation so using radiation therapy before or after removing the cancer is not routinely recommended because studies have shown no improvement in survival rates.

[0015] Renal cell cancers are very resistant to present forms of chemotherapy. Some drugs, such as vinblastine, floxuridine, and 5-fluorouracil (5-FU) are mildly effective. A combination of 5-FU and gemcitabine has benefited some patients. A 5-FU-like drug, capecitabine, may also have some benefit.

[0016] Cytokines (interleukin-2 (IL-2) and interferon-alpha) have become one of the standard treatments for metastatic renal cell carcinoma. These cause the cancers to shrink to less than half their original size in about 10% to 20% of patients. Patients who respond to IL-2 tend to have lasting responses. Recent research with a combination of IL-2, interferon, and chemotherapy (using 5-fluorouracil) is also promising and may offer a better chance of partial or complete remission. Cytokine therapy does have severe side effects however.

[0017] Sorafenib (Nexavar), Sunitinib (Sutent) and Bevacizumab (Avastin) are other drugs which may also be effective against renal cell cancer.

Kidney Cancer Survival by Stage

[0018]

T stage cancer	5/10-year cancer-specific survival
T1	95%/91%
T2	80%/70%
T3a	66%/53%
T3b	52%/43%
T3c	43%/42%

Lung Cancer

[0019] Lung cancer is the most common form of cancer worldwide (accounting for about 12% of cancer cases) and the main cause of death from cancer (accounting for about 18% of deaths). Global incidence of lung cancer is over 1,300,000 per year, with the number of deaths over 1,100,000. In the USA, there are about 170,000 new cases per year (about

13% of all cancers), with about 160,000 deaths (about 28% of cancer deaths). Lung cancer is much more prevalent among men than women. Nearly 70% of people diagnosed with lung cancer are older than 65; fewer than 3% of all cases are found in people under the age of 45. Around 15% of all lung cancers are small cell type (SCLC), which tend to spread widely through the body, while the remaining 85% are non-small cell (NSCLC). It has been estimated that approximately US \$9.6 billion is spent in the USA each year on treating lung cancer.

Lung Cancer Diagnosis

[0020] Lung cancer is a life-threatening disease because it often metastasises even before it can be detected on a chest x-ray. Usually symptoms of lung cancer do not appear until the disease is in an advanced stage. So far, there is no screening test that has been shown to improve a person's chance for a cure. Imaging tests such as a chest x-ray, CT scan, MRI scan or PET scan may be used to detect lung cancer. Tests to confirm the diagnosis are then performed and include sputum cytology, needle biopsy, bronchoscopy, endobronchial ultrasound and complete blood count (CBC).

Lung Cancer Staging

[0021] Nearly 60% of people diagnosed with lung cancer die within one year of diagnosis; 75% die within 2 years. The 5-year survival rate for people diagnosed with NSCLC is about 15%; for SCLC the 5-year survival rate is about 6%. NSCLC is staged using the American Joint Committee on Cancer (AJCC) TNM system—Stage 0—Stage IV. The 5-year survival rates by stage are as follows: stage I: 47%; stage II: 26%; stage III: 8% and stage IV: 2%. SCLC has a 2-stage system—limited stage and extensive stage. About two thirds of SCLC patients have extensive disease at diagnosis. If SCLC is found very early and is localised to the lung alone, the 5-year survival rate is around 21%, but only 6% of patients fall into this category. Where the cancer has spread, the 5-year survival is around 11%. For patients with extensive disease, the 5-year survival is just 2%.

Lung Cancer Treatment

[0022] Surgery is the only reliable method to cure NSCLC. Types of surgery include lobectomy, pneumonectomy, segmentectomy and video-assisted thoracic surgery (for small tumours). External beam radiation therapy is sometimes used as the primary treatment, especially if the patient's health is too poor to undergo surgery. Radiation therapy can also be used after surgery. Chemotherapy may be given as the primary treatment or as an adjuvant to surgery. Targeted therapy using epidermal growth factor receptor (EGFR) antagonists such as gefitinib or erlotinib can also be given after other treatments have failed. Antiangiogenic drugs, such as bevacizumab, have been found to prolong survival of patients with advanced lung cancer. Photodynamic therapy is also being researched as a treatment for lung cancer.

[0023] The main treatment for SCLC is chemotherapy, either alone or in combination with external beam radiation therapy and very rarely, surgery.

[0024] Chemotherapeutic agents used for NSCLC and SCLC include cisplatin, carboplatin, mitomycin C, ifosfamide, vinblastine, gemcitabine, etoposide, vinorelbine, paclitaxel, docetaxel and irinotecan.

Pancreatic Cancer

[0025] Pancreatic cancer is a very difficult cancer to detect and the prognosis for patients is usually very poor. The num-

ber of new cases and deaths per year is almost equal. Global incidence of pancreatic cancer is approximately 230,000 cases (about 2% of all cancer cases), with about 225,000 deaths (3.4% of cancer deaths) per year. It is much more prevalent in the developed world. In the USA, there are about 34,000 new cases per year, with about 32,000 deaths. It has been estimated that approximately US \$1.5 billion is spent in the USA each year on treating pancreatic cancer.

Pancreatic Cancer Diagnosis

[0026] Pancreatic cancer is very difficult to detect and very few pancreatic cancers are found early. Patients usually have no symptoms until the cancer has spread to other organs. There are currently no blood tests or easily available screening tests that can accurately detect early cancers of the pancreas. An endoscopic ultrasound followed by a biopsy is the best way to diagnose pancreatic cancer. Other detection methods include CT, CT-guided needle biopsy, PET, ultrasonography and MRI. Blood levels of CA 19-9 and carcinoembryonic antigen (CEA) may be elevated but by the time blood levels are high enough to be detected, the cancer is no longer in its early stages.

Pancreatic Cancer Staging

[0027] Pancreatic cancer has four stages, stage I to stage IV according to the American Joint Committee on Cancer (AJCC) TNM system. Pancreatic cancer is also divided into resectable, locally advanced (unresectable) and metastatic cancer. For patients with advanced cancers, the overall survival rate is <1% at 5 years with most patients dying within 1 year.

Pancreatic Cancer Treatment

[0028] Surgery is the only method of curing pancreatic cancer. About 10% of pancreatic cancers are contained entirely within the pancreas at the time of diagnosis and attempts to remove the entire cancer by surgery may be successful in some of these patients. The 5-year survival for those undergoing surgery with the intent of completely removing the cancer is about 20%. Potentially curative surgery, usually by pancreaticoduodenectomy (Whipple procedure), is used when it may be possible to remove all of the cancer. Palliative surgery may be performed if the tumour is too widespread to be completely removed. Removing only part of the cancer does not allow patients to live longer. Pancreatic cancer surgery is difficult to perform with a high likelihood of complications.

[0029] External beam radiation therapy combined with chemotherapy can be given before or after surgery and can also be given to patients whose tumours are too widespread to be removed by surgery. The main chemotherapeutic agents which are used are gemcitabine and 5-fluorouracil. Targeted therapy using drugs such as erlotinib and cetuximab may be of benefit to patients with advanced pancreatic cancer.

Therapeutic Challenges

[0030] The major challenges in treatment of the above mentioned cancers are to improve early detection rates, to find new non-invasive markers that can be used to follow disease progression and identify relapse, and to find improved and less toxic therapies, especially for more advanced disease where 5 year survival is still poor. There is a great need to identify targets which are more specific to the cancer cells,

e.g. ones which are expressed on the surface of the tumour cells so that they can be attacked by promising new approaches like immunotherapeutics and targeted toxins.

SUMMARY OF THE INVENTION

[0031] The present invention provides methods and compositions for therapy, screening, diagnosis and prognosis of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, for colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer stratification, for monitoring the effectiveness of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer treatment, and for drug development for treatment of one or more of same.

[0032] We have used mass spectrometry to identify peptides generated by gel electrophoresis or tagging with iTRAQ reagents and tryptic digest of membrane proteins extracted from colorectal, kidney, lung and pancreatic cancer tissue samples. Peptide sequences were compared to existing protein and cDNA databases and the corresponding gene sequences identified.

[0033] A first aspect of the invention provides an OGTA066 polypeptide, one or more immunogenic fragments or derivatives thereof for use in the treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer or use of same in treatment or prophylaxis of said cancers.

[0034] In a second aspect the invention provides methods of treating colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, comprising administering to a patient a therapeutically effective amount of a compound that modulates (e.g., upregulates or downregulates) or complements the expression or the biological activity (or both) of one or more of the proteins of the invention in patients having colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, in order to (a) prevent the onset or development of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer (b) prevent the progression of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer or (c) ameliorate the symptoms of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer.

[0035] According to a third aspect of the invention we provide a method of detecting, diagnosing and/or screening for or monitoring the progression of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or of monitoring the effect of an anti-colorectal cancer, anti-kidney cancer, anti-lung cancer or anti-pancreatic cancer drug or therapy in a subject which comprises detecting the presence or level of OGTA066, or one or more fragments thereof, or the presence or level of nucleic acid encoding OGTA066 or the level of the activity of OGTA066 or which comprises detecting a change in any of the aforementioned in said subject, for example in comparison to a health individual.

[0036] According to a fourth aspect of the invention we provide a method of detecting, diagnosing and/or screening for colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in a candidate subject which comprises detecting the presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066 in said candidate subject, in which either (a) the presence of an elevated level of OGTA066 or said one or more fragments thereof or an elevated level of nucleic acid encoding OGTA066 or the

presence of an elevated level of OGTA066 activity in the candidate subject as compared with the level in a healthy subject or

[0037] (b) the presence of a detectable level of OGTA066 or said one or more fragments thereof or a detectable level of nucleic acid encoding OGTA066 or the presence of a detectable level of OGTA066 activity in the candidate subject as compared with a corresponding undetectable level in a healthy subject

indicates the presence of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in said subject.

[0038] According to a fifth aspect of the invention we provide a method of monitoring the progression of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in a subject or of monitoring the effect of an anti-colorectal cancer, anti-kidney cancer, anti-lung cancer or anti-pancreatic cancer drug or therapy which comprises detecting the presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066 in said candidate subject at a first time point and at a later time point, the presence of an elevated or lowered level of OGTA066 or said one or more fragments thereof or an elevated or lowered level of nucleic acid encoding OGTA066 or the presence of an elevated or lowered level of OGTA066 activity in the subject at the later time point as compared with the level in the subject at said first time point, indicating the progression or regression of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or indicating the effect or non-effect of an anti-colorectal cancer, anti-kidney cancer, anti-lung cancer or anti-pancreatic cancer drug or therapy in said subject.

[0039] The presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066 may, for example, be detected by analysis of a biological sample obtained from said subject.

[0040] The method of invention may typically include the step of obtaining a biological sample for analysis from said subject. However, in one or more aspects the sampling step does not form part of the method(s) according to the invention.

[0041] The biological sample used can be from any source such as a serum sample or a tissue sample e.g. colorectal, kidney, lung or pancreatic tissue. For instance, when looking for evidence of metastatic colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, one would look at major sites of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer metastasis, e.g. the liver, the peritoneal cavity, the pelvis, the retroperitoneum and the lungs for colorectal cancer, the bones, the lungs and the liver for kidney cancer; the brain, the liver, the bones and adrenal glands for lung cancer and the liver for pancreatic cancer.

[0042] Alternatively the presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066 may be detected by analysis in situ.

[0043] In certain embodiments, methods of diagnosis described herein may be at least partly, or wholly, performed in vitro.

[0044] Suitably the presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066 is detected quantitatively.

[0045] For example, quantitatively detecting may comprise:

[0046] (a) contacting a biological sample with an affinity reagent that is specific for OGTA066, said affinity reagent optionally being conjugated to a detectable label; and

[0047] (b) detecting whether binding has occurred between the affinity reagent and at least one species in the sample, said detection being performed either directly or indirectly.

[0048] Alternatively the presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066 may be detected quantitatively by means involving use of an imaging technology.

[0049] In another embodiment, the method of the invention involves use of immunohistochemistry on tissue sections in order to determine the presence of OGTA066, or one or more fragments thereof, or the presence of nucleic acid encoding OGTA066 or the presence of the activity of OGTA066, and thereby to localise colorectal cancer, kidney cancer, lung cancer or pancreatic cancer cells.

[0050] In one embodiment the presence of OGTA066 or one or more epitope-containing fragments thereof is detected, for example using an affinity reagent capable of specific binding to OGTA066 or one or more fragments thereof, such as an antibody.

[0051] In another embodiment the activity of OGTA066 is detected.

[0052] According to another aspect of the invention there is provided a method of detecting, diagnosing and/or screening for or monitoring the progression of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or of monitoring the effect of an anti-colorectal cancer, anti-kidney cancer, anti-lung cancer or anti-pancreatic cancer drug or therapy in a subject which comprises detecting the presence or level of antibodies capable of immunospecific binding to OGTA066, or one or more epitope-containing fragments thereof or which comprises detecting a change in the level thereof in said subject.

[0053] According to another aspect of the invention there is also provided a method of detecting, diagnosing and/or screening for colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in a subject which comprises detecting the presence of antibodies capable of immunospecific binding to OGTA066, or one or more epitope-containing fragments thereof in said subject, in which (a) the presence of an elevated level of antibodies capable of immunospecific binding to OGTA066 or said one or more epitope-containing fragments thereof in said subject as compared with the level in a healthy subject or (b) the presence of a detectable level of antibodies capable of immunospecific binding to OGTA066 or said one or more epitope-containing fragments thereof in said subject as compared with a corresponding undetectable level in a healthy subject indicates the presence of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in said subject.

[0054] One particular method of detecting, diagnosing and/or screening for colorectal cancer, kidney cancer, lung cancer or pancreatic cancer comprises:

[0055] (a) bringing into contact with a biological sample to be tested OGTA066, or one or more epitope-containing fragments thereof; and

[0056] (b) detecting the presence of antibodies in the subject capable of immunospecific binding to OGTA066, or one or more epitope-containing fragments thereof.

[0057] According to another aspect of the invention there is provided a method of monitoring the progression of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or of monitoring the effect of an anti-colorectal cancer, anti-kidney cancer, anti-lung cancer or anti-pancreatic cancer drug or therapy in a subject which comprises detecting the presence of antibodies capable of immunospecific binding to OGTA066, or one or more epitope-containing fragments thereof in said subject at a first time point and at a later time point, the presence of an elevated or lowered level of antibodies capable of immunospecific binding to OGTA066, or one or more epitope-containing fragments thereof in said subject at the later time point as compared with the level in said subject at said first time point, indicating the progression or regression of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or the effect or non-effect of an anti-colorectal cancer, anti-kidney cancer, anti-lung cancer or anti-pancreatic cancer drug or therapy in said subject.

[0058] The presence of antibodies capable of immunospecific binding to OGTA066, or one or more epitope-containing fragments thereof is typically detected by analysis of a biological sample obtained from said subject (exemplary biological samples are mentioned above, e.g. the sample is a sample of colorectal, kidney, lung or pancreatic tissue, or else a sample of blood or saliva).

[0059] The method typically includes the step of obtaining said biological sample for analysis from said subject.

[0060] The antibodies that may be detected include IgA, IgM and IgG antibodies.

[0061] In any of the above methods, the level that may be detected in the candidate subject who has colorectal cancer, kidney cancer, lung cancer or pancreatic cancer is 2 or more fold higher than the level in the healthy subject.

[0062] Another aspect of the invention is an agent capable of specific binding to OGTA066, or a fragment thereof, or a hybridising agent capable of hybridizing to nucleic acid encoding OGTA066 or an agent capable of detecting the activity of OGTA066 for use in screening for, detecting and/or diagnosing disease, such as cancer, and especially colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0063] Another aspect of the invention is OGTA066, or a fragment thereof for use in screening for, detecting and/or diagnosing disease such as cancer, and especially colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0064] Another aspect of the invention is an affinity reagent capable of specific binding to OGTA066 or a fragment thereof, for example an affinity reagent which contains or is conjugated to a detectable label or contains or is conjugated to a therapeutic moiety such as a cytotoxic moiety. The affinity reagent may, for example, be an antibody.

[0065] Another aspect of the invention is a hybridizing agent capable of hybridizing to nucleic acid encoding OGTA066, for example, a hybridizing agent which contains or is conjugated to a detectable label. One example of a hybridizing agent is an inhibitory RNA (RNAi). Other examples include anti-sense oligonucleotides and ribozymes.

[0066] The invention also provides a kit containing OGTA066 and/or one or more fragments thereof or containing one or more aforementioned affinity reagents and/or hybridizing agents or containing one or more agents capable

of detecting the activity of OGTA066 together with instructions for their use in an aforementioned method. The kit may further contain reagents capable of detecting and reporting the binding of said affinity reagents and/or hybridizing agents to their binding partners.

[0067] Another aspect of the invention is a pharmaceutical composition comprising a therapeutically effective amount of an affinity reagent capable of specific binding to OGTA066 or a fragment thereof.

[0068] Another aspect of the invention is a pharmaceutically acceptable diluent or carrier and a pharmaceutical composition comprising one or more affinity reagents or hybridizing reagents as aforesaid and a pharmaceutically acceptable diluent or carrier.

[0069] In another embodiment the cancer to be detected, prevented or treated is colorectal cancer.

[0070] In another embodiment the cancer to be detected, prevented or treated is kidney cancer.

[0071] In another embodiment the cancer to be detected, prevented or treated is lung cancer.

[0072] In another embodiment the cancer to be detected, prevented or treated is pancreatic cancer.

[0073] Other aspects of the present invention are set out below and in the claims herein.

BRIEF DESCRIPTION OF THE FIGURES

[0074] FIG. 1 shows the amino acid sequence of the protein of the invention. The tryptic peptides detected experimentally by mass spectrometry are highlighted—mass match peptides are shown in bold, tandem peptides are underlined.

[0075] FIG. 2 shows the Protein Index for the protein of the invention.

[0076] FIGS. 3 and 5 show so-called Box Plot data for OGTA066 as described in Examples 3 and 4.

[0077] FIG. 4 shows ROC curve data for OGTA066 as described in Example 4.

DETAILED DESCRIPTION OF THE INVENTION

[0078] The invention described in detail below provides methods and compositions for clinical screening, diagnosis and prognosis of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in a mammalian subject for identifying patients most likely to respond to a particular therapeutic treatment, for monitoring the results of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer therapy, for drug screening and drug development. The invention also encompasses the administration of therapeutic compositions to a mammalian subject to treat or prevent colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer.

[0079] In one aspect the invention provides use of an OGTA066 polypeptide, or one or more fragments or derivatives thereof, for the treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer.

[0080] The invention also provides use of an OGTA066 polypeptide, one or more fragments or derivatives thereof in the manufacture of a medicament for the treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0081] In one aspect there is provided a method of treatment comprising administering a therapeutically effective amount of an OGTA066 polypeptide, one or more fragments or derivatives thereof, or one or more fragments or derivatives

thereof, for the treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer.

[0082] The invention further provides a method for the treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer in a subject, or of vaccinating a subject against colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, which comprises the step of administering to the subject an effective amount of an OGTA066 polypeptide and/or one or more antigenic or immunogenic fragments thereof, for example as a vaccine.

[0083] The mammalian subject may be a non-human mammal, but is generally human, such as a human adult, i.e. a human subject at least 21 (for example at least 35, at least 50, at least 60, at least 70, or at least 80) years old.

[0084] In one aspect there is provided a composition capable of eliciting an immune response in a subject, which composition comprises an OGTA066 polypeptide and/or one or more antigenic or immunogenic fragments thereof, and one or more suitable adjuvants (suitable adjuvants are discussed below).

[0085] The composition capable of eliciting an immune response may for example be provided as a vaccine comprising an OGTA066 polypeptide or derivatives thereof, and/or one or more antigenic or immunogenic fragments thereof.

[0086] In one aspect the invention provides an antibody or affinity agent/reagent capable of specifically binding to OGTA066 or a fragment thereof, for example for use in treatment or prophylaxis, such as treatment or prophylaxis of cancer, particularly a cancer as described herein.

OGTA066

[0087] In one aspect of the invention, 1D gel electrophoresis or isobaric tags for relative and absolute quantification (iTRAQ) or other appropriate methods are used to analyze colorectal cancer, kidney cancer, lung cancer or pancreatic cancer tissue samples from a subject, preferably a living subject, in order to measure the expression of the protein of the invention for screening or diagnosis of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, to determine the prognosis of a colorectal cancer, kidney cancer, lung cancer or pancreatic cancer patient, to monitor the effectiveness of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer therapy, or for drug development.

[0088] As used herein, the term "Protein of the invention", or "OGTA066", refers to the protein illustrated in FIG. 1 detected experimentally by 1D gel electrophoresis and iTRAQ analysis of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer tissue samples.

[0089] OGTA066 has been identified in membrane protein extracts of colorectal cancer, kidney cancer, lung cancer and pancreatic cancer tissue samples from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer patients, through the methods and apparatus of the Preferred Technologies described in Examples 1 and 2 (1D gel electrophoresis or iTRAQ together with tryptic digest of membrane protein extracts). Peptide sequences were compared to the SWISS-PROT and trEMBL databases (held by the Swiss Institute of Bioinformatics (SIB) and the European Bioinformatics Institute (EBI) which are available at www.expasy.com), and the following entry: □8TD06, Anterior gradient protein 3 homolog, was identified (also referred to as hAG-3 or BCMP11).

[0090] The full length protein is 166 amino acids in length with a sequence as shown in FIG. 1. The signal sequence of the molecule is found in amino acids 1 to 21.

[0091] Proteins employed in the invention are useful as are fragments e.g. antigenic or immunogenic fragments thereof and derivatives thereof. Epitope containing fragments including antigenic or immunogenic fragments will typically be of length 12 amino acids or more e.g. 20 amino acids or more e.g. 50 or 100 amino acids or more. Fragments may be 95% or more of the length of the full protein e.g. 90% or more e.g. 75% or 50% or 25% or 10% or more of the length of the full protein.

[0092] Alternatively, the proteins/polypeptides employed or referred to herein may be limited to those specifically recited/described in the present specification or a moiety 80, 85, 90, 91, 92, 93, 94, 95, 96, 97, 98 or 99% identical or similar thereto.

[0093] Epitope containing fragments including antigenic or immunogenic fragments will be capable of eliciting a relevant immune response in a patient. DNA encoding the protein employed in the invention may also be useful as are fragments thereof e.g. DNA encoding fragments of the protein employed in the invention such as immunogenic fragments thereof. Fragments of nucleic acid (e.g. DNA) encoding the protein employed in the invention may be 95% or more of the length of the full coding region e.g. 90% or more e.g. 75% or 50% or 25% or 10% or more of the length of the full coding region. Fragments of nucleic acid (e.g. DNA) may be 36 nucleotides or more e.g. 60 nucleotides or more e.g. 150 or 300 nucleotides or more in length.

[0094] Derivatives of the protein employed in the invention include variants on the sequence in which one or more (e.g. 1-20 such as 15, or up to 20% such as up to 10% or 5% or 1% by number based on the total length of the protein) deletions, insertions or substitutions have been made. Substitutions may typically be conservative substitutions. Derivatives of the protein employed in the invention include variants on the sequence which have a sequence identity over the entire length thereof of typically at least 60%, 70%, 80%, 90 or 95%. Derivatives will typically have essentially the same biological function as the protein from which they are derived. Derivatives will typically be comparably antigenic or immunogenic to the protein from which they are derived.

[0095] Derivatives of proteins also include chemically treated protein such as carboxymethylated, carboxyamidated or acetylated proteins, for example treated during purification.

[0096] In one aspect the invention relates to colorectal cancer.

[0097] In one aspect the invention relates to kidney cancer.

[0098] In one aspect the invention relates to lung cancer.

[0099] In one aspect the invention relates to pancreatic cancer.

[0100] Tables 1a and 1b below illustrate the different occurrences of OGTA066 as detected by 1D gel electrophoresis and mass spectrometry of membrane protein extracts of colorectal and pancreatic tissue samples from colorectal cancer and pancreatic cancer patients respectively. The first column provides the molecular weight and the last column provides a list of the sequences observed by mass spectrometry and the corresponding SEQ ID Nos.

[0101] Tables 2a-2c below illustrate the different occurrences of OGTA066 as detected by iTRAQ and mass spectrometry of membrane protein extracts of colorectal, kidney

and lung tissue samples from colorectal cancer, kidney cancer and lung cancer patients respectively. The first column provides the samples batch number, the second column gives the iTRAQ experiment number and the last column provides a list of sequences observed by mass spectrometry and the corresponding SEQ ID Nos.

TABLE 1a

<u>Colorectal cancer 1D gel</u>	
MW (Da)	Tryptics identified [SEQ ID No]
19046	IMFVDPSLTVR [3]
19131	IMFVDPSLTVR [3]
19217	IMFVDPSLTVR [3]
19304	IMFVDPSLTVR [3]

TABLE 1b

<u>Pancreatic cancer 1D gel</u>	
MW (Da)	Tryptics identified [SEQ ID No]
24387	IMFVDPSLTVR [3]
24611	IMFVDPSLTVR [3], KPLMVIHHLEDCQYSQALK [4]

TABLE 2a

<u>Colorectal cancer iTRAQ</u>		
Samples batch no.	Experiment no.	Tryptics identified [SEQ ID No]
Samples 1	Experiment 1	IMFVDPSLTVR [3]
Samples 1	Experiment 2	IMFVDPSLTVR [3]
Samples 2	Experiment 1	IMFVDPSLTVR [3]
Samples 2	Experiment 2	DLPLLIENMK [2], IMFVDPSLTVR [3]
Samples 2	Experiment 3	IMFVDPSLTVR [3]
Samples 2	Experiment 4	DLPLLIENMK [2], IMFVDPSLTVR [3]
Samples 2	Experiment 5	IMFVDPSLTVR [3]

TABLE 2b

<u>Kidney cancer iTRAQ</u>		
Sample no.	Experiment no.	Tryptics identified [SEQ ID No]
Samples 1	Experiment 1	DLPLLIENMK [2]

TABLE 2c

<u>Non-small cell lung cancer iTRAQ</u>		
Sample no.	Experiment no.	Tryptics identified [SEQ ID No]
Samples 1	Experiment 1	DLPLLIENMK [2], IMFVDPSLTVR [3], NLSPDGQYVPR [5]
Samples 2	Experiment 1	IMFVDPSLTVR [3]
Samples 2	Experiment 2	IMFVDPSLTVR [3]
Samples 2	Experiment 3	IMFVDPSLTVR [3]
Samples 2	Experiment 4	IMFVDPSLTVR [3]

[0102] For OGTA066, the detected level obtained upon analyzing tissue from subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer relative to the detected level obtained upon analyzing tissue from subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer will depend upon the particular analytical protocol and detection technique that is used. Accordingly, the present invention contemplates that each laboratory will establish a reference range in subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer according to the analytical protocol and detection technique in use, as is conventional in the diagnostic art. Preferably, at least one control positive tissue sample from a subject known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or at least one control negative tissue sample from a subject known to be free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer (and more preferably both positive and negative control samples) are included in each batch of test samples analysed.

[0103] OGTA066 can be used for detection, prognosis, diagnosis, or monitoring of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or for drug development. In one embodiment of the invention, tissue from a subject (e.g. a subject suspected of having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer) is analysed by 1D gel electrophoresis or iTRAQ for detection of OGTA066. An increased abundance of OGTA066 in the tissue from the subject relative to tissue from a subject or subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer (e.g. a control sample) or a previously determined reference range indicates the presence of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0104] OGTA066 may, in particular, be characterized as an isoform having a MW substantially as recited (e.g. +/-10%, particularly +/-5% of the value) in column 1 of any of the rows of Tables 1a-1b.

[0105] In one or more aspects the invention does not employ the full length protein of 166 amino acids.

[0106] In one or more aspects, for example in relation to colorectal cancer, kidney cancer, lung cancer or pancreatic cancer the polypeptide employed may be the full length protein.

[0107] Thus OGTA066 may refer to full length protein or 1, 2, 3 or 4 sequences selected from Sequence ID Nos: 2, 3, 4, 5.

[0108] The sequences shown in tables 1 or 2 may be employed in any relevant aspect of the invention.

[0109] In relation to fragments, immunogenic fragments or antigenic fragments of OGTA066:

[0110] for colorectal cancer applications: in one aspect of the invention these comprise one or more of the sequences identified as tryptic sequences in the 2nd column of Table 1a or the 3rd column of Table 2a;

[0111] for kidney cancer applications: in one aspect of the invention these comprise one or more of the sequences identified as tryptic sequences in the 3rd column of Table 2b;

[0112] for lung cancer applications: in one aspect of the invention these comprise one or more of the sequences identified as tryptic sequences in the 3rd column of Table 2c;

[0113] for pancreatic cancer applications: in one aspect of the invention these comprise one or more of the sequences identified as tryptic sequences in the 2nd column of Table 1b.

[0114] The present invention may employ: (a) preparations comprising isolated OGTA066; (b) preparations comprising one or more fragments of OGTA066; and (c) antibodies or other affinity reagents that bind to OGTA066, to said fragments thereof or both.

[0115] As used herein OGTA066 is "isolated" when it is present in a preparation that is substantially free of contaminating proteins, i.e., a preparation in which less than 10% by weight (for example less than 5%, more such as less than 1%) of the total protein present is contaminating protein(s). A contaminating protein is a protein having a significantly different amino acid sequence from that of isolated OGTA066 as determined by mass spectral analysis. As used herein, a "significantly different" sequence is one that permits the contaminating protein to be resolved from OGTA066 by mass spectral analysis, performed according to the Reference Protocols described in Examples 1 and 2.

[0116] Thus in one aspect the invention provides a pharmaceutical composition for the treatment of colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer comprising a therapeutically effective amount of: an OGTA066 polypeptide (particularly those defined above) or an immunogenic fragment thereof and an adjuvant. The invention also provides use of same in treatment or prevention of cancers according to the invention or in the manufacture of a medicament for the treatment of the same.

[0117] OGTA066 can be assayed by any method known to those skilled in the art, including but not limited to, the Preferred Technologies described herein in Examples 1 and 2, kinase assays, enzyme assays, binding assays and other functional assays, immunoassays, and western blotting. In one embodiment, OGTA066 is separated on a 1-D gel by virtue of its MW and visualized by staining the gel. In one embodiment, OGTA066 is stained with a fluorescent dye and imaged with a fluorescence scanner. Sypro Red (Molecular Probes, Inc., Eugene, Oreg.) is a suitable dye for this purpose. A preferred fluorescent dye is disclosed in U.S. application Ser. No. 09/412,168, filed on Oct. 5, 1999, which is incorporated herein by reference in its entirety. In another embodiment, OGTA066 is analysed using isobaric tags for relative and absolute quantification (iTRAQ).

[0118] Alternatively, OGTA066 can be detected in an immunoassay. In one embodiment, an immunoassay is performed by contacting a sample from a subject to be tested with an anti-OGTA066 antibody (or other affinity reagent) under conditions such that binding (e.g. immunospecific

binding) can occur if OGTA066 is present, and detecting or measuring the amount of any binding (e.g. immunospecific binding) by the agent. OGTA066 binding agents can be produced by the methods and techniques taught herein.

[0119] OGTA066 may be detected by virtue of the detection of a fragment thereof e.g. an epitope containing (e.g. an immunogenic or antigenic) fragment thereof. Fragments may have a length of at least 10, more typically at least 20 amino acids e.g. at least 50 or 100 amino acids.

[0120] In one embodiment, binding of an affinity reagent (e.g. an antibody) in tissue sections can be used to detect aberrant OGTA066 localization or an aberrant level of OGTA066. In a specific embodiment, an antibody (or other affinity reagent) to OGTA066 can be used to assay a patient tissue (e.g. a colorectal, kidney, lung or pancreatic tissue) for the level of OGTA066 where an aberrant level of OGTA066 is indicative of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. As used herein, an "aberrant level" means a level that is increased compared with the level in a subject free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer or a reference level.

[0121] Any suitable immunoassay can be used, including, without limitation, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays and protein A immunoassays.

[0122] For example, OGTA066 can be detected in a fluid sample (e.g. blood, urine, or saliva) by means of a two-step sandwich assay. In the first step, a capture reagent (e.g. an anti-OGTA066 antibody or other affinity reagent) is used to capture OGTA066. The capture reagent can optionally be immobilized on a solid phase. In the second step, a directly or indirectly labeled detection reagent is used to detect the captured OGTA066. In one embodiment, the detection reagent is a lectin. Any lectin can be used for this purpose that preferentially binds to OGTA066 rather than to other isoforms that have the same core protein as OGTA066 or to other proteins that share the antigenic determinant recognized by the antibody. In a preferred embodiment, the chosen lectin binds OGTA066 with at least 2-fold greater affinity, more preferably at least 5-fold greater affinity, still more preferably at least 10-fold greater affinity, than to said other isoforms that have the same core protein as OGTA066 or to said other proteins that share the antigenic determinant recognized by the affinity reagent. Based on the present description, a lectin that is suitable for detecting OGTA066 can readily be identified by methods well known in the art, for instance upon testing one or more lectins enumerated in Table I on pages 158-159 of Sumar et al., *Lectins as Indicators of Disease-Associated Glycoforms*, In: Gabius H-J & Gabius S (eds.), 1993, *Lectins and Glycobiology*, at pp. 158-174 (which is incorporated herein by reference in its entirety). In an alternative embodiment, the detection reagent is an antibody (or other affinity reagent), e.g. an antibody that specifically (e.g. immunospecifically) detects other post-translational modifications, such as an antibody that immunospecifically binds to phosphorylated amino acids. Examples of such antibodies include those that bind to phosphotyrosine (BD Transduction Laboratories, catalog nos.: P11230-050/P11230-150; P11120; P38820; P39020), those that bind to phosphoserine

(Zymed Laboratories Inc., South San Francisco, Calif., catalog no. 61-8100) and those that bind to phosphothreonine (Zymed Laboratories Inc., South San Francisco, Calif., catalogue nos. 71-8200, 13-9200).

[0123] If desired, a gene encoding OGTA066, a related gene, or related nucleic acid sequences or subsequences, including complementary sequences, can also be used in hybridization assays. A nucleotide encoding OGTA066, or subsequences thereof comprising at least 8 nucleotides, preferably at least 12 nucleotides, and most preferably at least 15 nucleotides can be used as a hybridization probe. Hybridization assays can be used for detection, prognosis, diagnosis, or monitoring of conditions, disorders, or disease states, associated with aberrant expression of the gene encoding OGTA066, or for differential diagnosis of subjects with signs or symptoms suggestive of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. In particular, such a hybridization assay can be carried out by a method comprising contacting a subject's sample containing nucleic acid with a nucleic acid probe capable of hybridizing to a DNA or RNA that encodes OGTA066, under conditions such that hybridization can occur, and detecting or measuring any resulting hybridization.

[0124] Hence nucleic acid encoding OGTA066 (e.g. DNA or more suitably RNA) may be detected, for example, using a hybridizing agent capable of hybridizing to nucleic acid encoding OGTA066.

[0125] One such exemplary method comprises:

[0126] (a) contacting one or more oligonucleotide probes comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding OGTA066, with an RNA obtained from a biological sample from the subject or with cDNA copied from the RNA, wherein said contacting occurs under conditions that permit hybridization of the probe to the nucleotide sequence if present;

[0127] (b) detecting hybridization, if any, between the probe and the nucleotide sequence; and

[0128] (c) comparing the hybridization, if any, detected in step (b) with the hybridization detected in a control sample, or with a previously determined reference range.

[0129] The invention also provides diagnostic kits, comprising an anti-OGTA066 antibody (or other affinity reagent). In addition, such a kit may optionally comprise one or more of the following: (1) instructions for using the anti-OGTA066 affinity reagent for diagnosis, prognosis, therapeutic monitoring or any combination of these applications; (2) a labeled binding partner to the affinity reagent; (3) a solid phase (such as a reagent strip) upon which the anti-OGTA066 affinity reagent is immobilized; and (4) a label or insert indicating regulatory approval for diagnostic, prognostic or therapeutic use or any combination thereof. If no labeled binding partner to the affinity reagent is provided, the anti-OGTA066 affinity reagent itself can be labeled with a detectable marker, e.g. a chemiluminescent, enzymatic, fluorescent, or radioactive moiety.

[0130] The invention also provides a kit comprising a nucleic acid probe capable of hybridizing to nucleic acid, suitably RNA, encoding OGTA066. In a specific embodiment, a kit comprises in one or more containers a pair of primers (e.g. each in the size range of 6-30 nucleotides, more preferably 10-30 nucleotides and still more preferably 10-20 nucleotides) that under appropriate reaction conditions can

prime amplification of at least a portion of a nucleic acid encoding OGTA066, such as by polymerase chain reaction (see, e.g. Innis et al., 1990, PCR Protocols, Academic Press, Inc., San Diego, Calif.), ligase chain reaction (see EP 320, 308) use of Q β replicase, cyclic probe reaction, or other methods known in the art.

[0131] A kit can optionally further comprise a predetermined amount of OGTA066 or a nucleic acid encoding OGTA066, e.g. for use as a standard or control.

Use in Clinical Studies

[0132] The diagnostic methods and compositions of the present invention can assist in monitoring a clinical study, e.g. to evaluate drugs for therapy of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. In one embodiment, candidate molecules are tested for their ability to restore OGTA066 levels in a subject having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer to levels found in subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer or, in a treated subject, to preserve OGTA066 levels at or near non-colorectal cancer, non-kidney cancer, non-lung cancer or non-pancreatic cancer values.

[0133] In another embodiment, the methods and compositions of the present invention are used to screen candidates for a clinical study to identify individuals having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer; such individuals can then be excluded from the study or can be placed in a separate cohort for treatment or analysis.

Production of Protein Employed in the Invention and Corresponding Nucleic Acid

[0134] In one aspect the invention provides a method of treating or preventing colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of nucleic acid encoding OGTA066 or one or more fragments or derivatives thereof, for example in the form of a vaccine.

[0135] In another aspect there is provided a method of treating or preventing colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer comprising administering to a subject in need of such treatment or prevention a therapeutically effective amount of nucleic acid that inhibits the function or expression of OGTA066.

[0136] These methods (and/or other DNA aspects disclosed herein) of the invention may, for example include wherein the nucleic acid is an OGTA066 anti-sense nucleic acid or ribozyme.

[0137] Thus the invention includes nucleic acid encoding OGTA066 or one or more fragments or derivatives thereof, for use in treating or preventing colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer or in the manufacture of a medicament for the treatment or prevention of said cancers.

[0138] There is also provided nucleic acid that inhibits the function or expression of OGTA066 for use in treating or preventing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or in the manufacture of a medicament for treatment or prevention of said cancers.

[0139] A DNA employed in the present invention can be obtained by isolation as a cDNA fragment from cDNA libraries using as starter materials commercial mRNAs and determining and identifying the nucleotide sequences thereof.

That is, specifically, clones are randomly isolated from cDNA libraries, which are prepared according to Ohara et al's method (DNA Research Vol. 4, 53-59 (1997)). Next, through hybridization, duplicated clones (which appear repeatedly) are removed and then in vitro transcription and translation are carried out. Nucleotide sequences of both termini of clones, for which products of 50 kDa or more are confirmed, are determined.

[0140] Furthermore, databases of known genes are searched for homology using the thus obtained terminal nucleotide sequences as queries.

[0141] In addition to the above screening method, the 5' and 3' terminal sequences of cDNA are related to a human genome sequence. Then an unknown long-chain gene is confirmed in a region between the sequences, and the full-length of the cDNA is analyzed. In this way, an unknown gene that is unable to be obtained by a conventional cloning method that depends on known genes can be systematically cloned.

[0142] Moreover, all of the regions of a human-derived gene containing a DNA of the present invention can also be prepared using a PCR method such as RACE while paying sufficient attention to prevent artificial errors from taking place in short fragments or obtained sequences. As described above, clones having DNA of the present invention can be obtained.

[0143] In another means for cloning DNA employed in the present invention, a synthetic DNA primer having an appropriate nucleotide sequence of a portion of a polypeptide of the present invention is produced, followed by amplification by the PCR method using an appropriate library. Alternatively, selection can be carried out by hybridization of the DNA of the present invention with a DNA that has been incorporated into an appropriate vector and labeled with a DNA fragment or a synthetic DNA encoding some or all of the regions of the polypeptide of the present invention. Hybridization can be carried out by, for example, the method described in Current Protocols in Molecular Biology (edited by Frederick M. Ausubel et al., 1987). DNA of the present invention may be any DNA, as long as they contain nucleotide sequences encoding the polypeptides of the present invention as described above. Such a DNA may be a cDNA identified and isolated from cDNA libraries or the like that are derived from colorectal, kidney, lung or pancreatic tissue. Such a DNA may also be a synthetic DNA or the like. Vectors for use in library construction may be any of bacteriophages, plasmids, cosmids, phagemids, or the like. Furthermore, by the use of a total RNA fraction or a mRNA fraction prepared from the above cells and/or tissues, amplification can be carried out by a direct reverse transcription coupled polymerase chain reaction (hereinafter abbreviated as "RT-PCR method").

[0144] DNA encoding the above polypeptides consisting of amino acid sequences that are substantially identical to the amino acid sequences of OGTA066, or DNA encoding the above polypeptide consisting of an amino acid sequence derived from the amino acid sequence of OGTA066 by deletion, substitution, or addition of one or more amino acids composing a portion of the amino acid sequence can be easily produced by an appropriate combination of, for example, a site-directed mutagenesis method, a gene homologous recombination method, a primer elongation method, and the PCR method known by persons skilled in the art. In addition, at this time, a possible method for causing a polypeptide to have substantially equivalent biological activity is substitution of homologous amino acids (e.g. polar and nonpolar

amino acids, hydrophobic and hydrophilic amino acids, positively-charged and negatively charged amino acids, and aromatic amino acids) among amino acids composing the polypeptide. Furthermore, to maintain substantially equivalent biological activity, amino acids within functional domains contained in each polypeptide of the present invention are preferably conserved.

[0145] Furthermore, examples of DNA of the present invention include DNA comprising a nucleotide sequence that encodes the amino acid sequence of OGTA066 and DNA hybridizing under stringent conditions to the DNA and encoding a polypeptide (protein) having biological activity (function) equivalent to the function of the polypeptide consisting of the amino acid sequence of OGTA066. Under such conditions, an example of such DNA capable of hybridizing to DNA comprising the nucleotide sequence that encodes the amino acid sequence of OGTA066 is DNA comprising a nucleotide sequence that has a degree of overall mean homology with the entire nucleotide sequence of the DNA, such as approximately 80% or more, for example approximately 90% or more, such as approximately 95% or more. Hybridization can be carried out according to a method known in the art such as a method described in Current Protocols in Molecular Biology (edited by Frederick M. Ausubel et al., 1987) or a method according thereto. Here, "stringent conditions" are, for example, conditions of approximately "1*SSC, 0.1% SDS, and 37° C., more stringent conditions of approximately "0.5*SSC, 0.1% SDS, and 42° C., or even more stringent conditions of approximately "0.2*SSC, 0.1% SDS, and 65° C. With more stringent hybridization conditions, the isolation of a DNA having high homology with a probe sequence can be expected. The above combinations of SSC, SDS, and temperature conditions are given for illustrative purposes. Stringency similar to the above can be achieved by persons skilled in the art using an appropriate combination of the above factors or other factors (for example, probe concentration, probe length, and reaction time for hybridization) for determination of hybridization stringency.

[0146] A cloned DNA of the present invention can be directly used or used, if desired, after digestion with a restriction enzyme or addition of a linker, depending on purposes. The DNA may have ATG as a translation initiation codon at the 5' terminal side and have TAA, TGA, or TAG as a translation termination codon at the 3' terminal side. These translation initiation and translation termination codons can also be added using an appropriate synthetic DNA adapter.

[0147] In the methods/uses of the invention OGTA066, may for example be provided in isolated form such as where the OGTA066 polypeptide has been purified to at least to some extent. OGTA066 polypeptide may be provided in substantially pure form, that is to say free, to a substantial extent, from other proteins. OGTA066 polypeptide can also be produced using recombinant methods, synthetically produced or produced by a combination of these methods. OGTA066 can be easily prepared by any method known by persons skilled in the art, which involves producing an expression vector containing appropriate DNA of the present invention or a gene containing a DNA, culturing a transformant transformed using the expression vector, generating and accumulating a relevant polypeptide or a recombinant protein containing the polypeptide, and then collecting the resultant.

[0148] Recombinant OGTA066 polypeptide may be prepared by processes well known in the art from genetically engineered host cells comprising expression systems.

Accordingly, the present invention also relates to expression systems which comprise an OGTA066 polypeptide or nucleic acid, to host cells which are genetically engineered with such expression systems and to the production of OGTA066 polypeptide by recombinant techniques. For recombinant OGTA066, polypeptide production, host cells can be genetically engineered to incorporate expression systems or portions thereof for nucleic acids. Such incorporation can be performed using methods well known in the art, such as, calcium phosphate transfection, DEAD-dextran mediated transfection, transfection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection (see e.g. Davis et al., *Basic Methods in Molecular Biology*, 1986 and Sambrook et al., *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbour laboratory Press, Cold Spring Harbour, N.Y., 1989).

[0149] As host cells, for example, bacteria of the genus *Escherichia*, *Streptococci*, *Staphylococci*, *Streptomyces*, bacteria of the genus *Bacillus*, yeast, *Aspergillus* cells, insect cells, insects, and animal cells are used. Specific examples of bacteria of the genus *Escherichia*, which are used herein, include *Escherichia coli* K12 and DH1 (Proc. Natl. Acad. Sci. U.S.A., Vol. 60, 160 (1968)), JM103 (Nucleic Acids Research, Vol. 9, 309 (1981)), JA221 (Journal of Molecular Biology, Vol. 120, 517 (1978)), and HB101 (Journal of Molecular Biology, Vol. 41, 459 (1969)). As bacteria of the genus *Bacillus*, for example, *Bacillus subtilis* MI114 (Gene, Vol. 24, 255 (1983)) and 207-21 (Journal of Biochemistry, Vol. 95, 87 (1984)) are used. As yeast, for example, *Saccharomyces cerevisiae* AH22, AH22R-, NA87-11A, DKD-5D, and 20B-12, *Schizosaccharomyces pombe* NCYC1913 and NCYC2036, and *Pichia pastoris* are used. As insect cells, for example, *Drosophila* S2 and *Spodoptera* Sf9 cells are used. As animal cells, for example, COS-7 and Vero monkey cells, CHO Chinese hamster cells (hereinafter abbreviated as CHO cells), dhfr-gene-deficient CHO cells, mouse L cells, mouse AtT-20 cells, mouse myeloma cells, rat GH3 cells, human FL cells, COS, HeLa, C127, 3T3, HEK 293, BHK and Bowes melanoma cells are used.

[0150] Cell-free translation systems can also be employed to produce recombinant polypeptides (e.g. rabbit reticulocyte lysate, wheat germ lysate, SP6/T7 in vitro T&T and RTS100 *E. Coli* HY transcription and translation kits from Roche Diagnostics Ltd., Lewes, UK and the TNT Quick coupled Transcription/Translation System from Promega UK, Southampton, UK).

[0151] The expression vector can be produced according to a method known in the art. For example, the vector can be produced by (1) excising a DNA fragment containing a DNA of the present invention or a gene containing a DNA of the present invention and (2) ligating the DNA fragment downstream of the promoter in an appropriate expression vector. A wide variety of expression systems can be used, such as and without limitation, chromosomal, episomal and virus-derived systems, e.g. plasmids derived from *Escherichia coli* (e.g. pBR322, pBR325, pUC18, and pUC118), plasmids derived from *Bacillus subtilis* (e.g. pUB110, pTP5, and pC194), from bacteriophage, from transposons, from yeast episomes (e.g. pSH19 and pSH15), from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those

derived from plasmid and bacteriophage (such as [lambda] phage) genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Promoters to be used in the present invention may be any promoters as long as they are appropriate for hosts to be used for gene expression. For example, when a host is *Escherichia coli*, a trp promoter, a lac promoter, a recA promoter, a pL promoter, an l pp promoter, and the like are preferred. When a host is *Bacillus subtilis*, an SPO1 promoter, an SPO2 promoter, a penP promoter, and the like are preferred. When a host is yeast, a PHOS promoter, a PGK promoter, a GAP promoter, an ADH promoter, and the like are preferred. When an animal cell is used as a host, examples of promoters for use in this case include an SRa promoter, an SV40 promoter, an LTR promoter, a CMV promoter, and an HSV-TK promoter. Generally, any system or vector that is able to maintain, propagate or express a nucleic acid to produce a polypeptide in a host may be used.

[0152] The appropriate nucleic acid sequence may be inserted into an expression system by any variety of well known and routine techniques, such as those set forth in Sambrook et al., supra. Appropriate secretion signals may be incorporated into the OGTA066 polypeptide to allow secretion of the translated protein into the lumen of the endoplasmic reticulum, the periplasmic space or the extracellular environment. These signals may be endogenous to the OGTA066 polypeptide or they may be heterologous signals. Transformation of the host cells can be carried out according to methods known in the art. For example, the following documents can be referred to: Proc. Natl. Acad. Sci. U.S.A., Vol. 69, 2110 (1972); Gene, Vol. 17, 107 (1982); Molecular & General Genetics, Vol. 168, 111 (1979); Methods in Enzymology, Vol. 194, 182-187 (1991); Proc. Natl. Acad. Sci. U.S.A., Vol. 75, 1929 (1978); Cell Technology, separate volume 8, New Cell Technology, Experimental Protocol. 263-267 (1995) (issued by Shujunsha); and Virology, Vol. 52, 456 (1973). The thus obtained transformant transformed with an expression vector containing a DNA of the present invention or a gene containing a DNA of the present invention can be cultured according to a method known in the art. For example, when hosts are bacteria of the genus *Escherichia*, the bacteria are generally cultured at approximately 15° C. to 43° C. for approximately 3 to 24 hours. If necessary, aeration or agitation can also be added. When hosts are bacteria of the genus *Bacillus*, the bacteria are generally cultured at approximately 30° C. to 40° C. for approximately 6 to 24 hours. If necessary, aeration or agitation can also be added. When transformants whose hosts are yeast are cultured, culture is generally carried out at approximately 20° C. to 35° C. for approximately 24 to 72 hours using media with pH adjusted to be approximately 5 to 8. If necessary, aeration or agitation can also be added. When transformants whose hosts are animal cells are cultured, the cells are generally cultured at approximately 30° C. to 40° C. for approximately 15 to 60 hours using media with the pH adjusted to be approximately 6 to 8. If necessary, aeration or agitation can also be added.

[0153] If an OGTA066 polypeptide is to be expressed for use in cell-based screening assays, it is preferred that the polypeptide be produced at the cell surface. In this event, the cells may be harvested prior to use in the screening assay. If the OGTA066 polypeptide is secreted into the medium, the medium can be recovered in order to isolate said polypeptide. If produced intracellularly, the cells must first be lysed before the OGTA066 polypeptide is recovered.

[0154] OGTA066 polypeptide can be recovered and purified from recombinant cell cultures or from other biological sources by well known methods including, ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, affinity chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography, molecular sieving chromatography, centrifugation methods, electrophoresis methods and lectin chromatography. In one embodiment, a combination of these methods is used. In another embodiment, high performance liquid chromatography is used. In a further embodiment, an antibody which specifically binds to an OGTA066 polypeptide can be used to deplete a sample comprising an OGTA066 polypeptide of said polypeptide or to purify said polypeptide.

[0155] To separate and purify a polypeptide or a protein of the present invention from the culture products, for example, after culture, microbial bodies or cells are collected by a known method, they are suspended in an appropriate buffer, the microbial bodies or the cells are disrupted by, for example, ultrasonic waves, lysozymes, and/or freeze-thawing, the resultant is then subjected to centrifugation or filtration, and then a crude extract of the protein can be obtained. The buffer may also contain a protein denaturation agent such as urea or guanidine hydrochloride or a surfactant such as Triton X-100™. When the protein is secreted in a culture solution, microbial bodies or cells and a supernatant are separated by a known method after the completion of culture and then the supernatant is collected. The protein contained in the thus obtained culture supernatant or the extract can be purified by an appropriate combination of known separation and purification methods. The thus obtained polypeptide (protein) of the present invention can be converted into a salt by a known method or a method according thereto. Conversely, when the polypeptide (protein) of the present invention is obtained in the form of a salt, it can be converted into a free protein or peptide or another salt by a known method or a method according thereto. Moreover, an appropriate protein modification enzyme such as trypsin or chymotrypsin is caused to act on a protein produced by a recombinant before or after purification, so that modification can be arbitrarily added or a polypeptide can be partially removed. The presence of a polypeptide (protein) of the present invention or a salt thereof can be measured by various binding assays, enzyme immunoassays using specific antibodies, and the like.

[0156] Techniques well known in the art, may be used for refolding to regenerate native or active conformations of the OGTA066 polypeptide when the polypeptide has been denatured during isolation and or purification. In the context of the present invention, OGTA066 polypeptide can be obtained from a biological sample from any source, such as and without limitation, a blood sample or tissue sample, e.g. colorectal, kidney, lung or pancreatic tissue.

[0157] OGTA066 polypeptide may be in the form of a "mature protein" or may be part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, a pre-, pro- or prepro-protein sequence, or a sequence which aids in purification such as an affinity tag, for example, but without limitation, multiple histidine residues, a FLAG tag, HA tag or myc tag.

[0158] OGTA066 or one or more sequences selected from Sequence ID Nos 1 to 5 may, for example be fused with a heterologous fusion partner such as the surface protein,

known as protein D from *Haemophilus Influenza B*, a non-structural protein from influenzae virus such as NS1, the S antigen from Hepatitis B or a protein known as LYTA such as the C terminal thereof.

[0159] An additional sequence that may provide stability during recombinant production may be used. Such sequences may be optionally removed as required by incorporating a cleavable sequence as an additional sequence or part thereof. Thus, an OGTA066 polypeptide may be fused to other moieties including other polypeptides or proteins (for example, glutathione S-transferase and protein A). Such a fusion protein can be cleaved using an appropriate protease, and then separated into each protein. Such additional sequences and affinity tags are well known in the art. In addition to the above, features known in the art, such as an enhancer, a splicing signal, a polyA addition signal, a selection marker, and an SV40 replication origin can be added to an expression vector, if desired.

Production of Affinity Reagents to OGTA066

[0160] There are three main types of immunoaffinity reagent—monoclonal antibodies, phage display antibodies and smaller antibody-derived molecules such as Affibodies, Domain Antibodies (dAbs), Nanobodies or UniBodies. In general in applications according to the present invention where the use of antibodies is stated, other affinity reagents (e.g. Affibodies, domain antibodies, Nanobodies or UniBodies) may be employed. Such substances may be said to be capable of immunospecific binding to OGTA066. Where appropriate the term "affinity agent" shall be construed to embrace immunoaffinity reagents and other substances capable of specific binding to OGTA066 including but not limited to ligands, lectins, streptavidins, antibody mimetics and synthetic binding agents.

[0161] In one aspect the invention provides an antibody or other affinity reagent capable of immunospecific binding to an OGTA066 polypeptide, for example a sequence as shown in Sequence ID No: 1, 2, 3, 4 or 5.

[0162] In one aspect the invention provides an affinity reagent as herein defined for the treatment or prevention of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or use of said affinity reagent for the manufacture of a medicament for the treatment of same.

Production of Antibodies to OGTA066

[0163] According to the invention OGTA066, an OGTA066 analog, an OGTA066-related protein or a fragment or derivative of any of the foregoing may be used as an immunogen to generate antibodies which immunospecifically bind such an immunogen. Such immunogens can be isolated by any convenient means, including the methods described above. The term "antibody" as used herein refers to a peptide or polypeptide derived from, modeled after or substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof, capable of specifically binding an antigen or epitope. See, e.g. *Fundamental Immunology*, 3rd Edition, W. E. Paul, ed., Raven Press, N. Y. (1993); Wilson (1994) *J. Immunol. Methods* 175:267-273; Yarmush (1992) *J. Biochem. Biophys. Methods* 25:85-97. The term antibody includes antigen-binding portions, i.e., "antigen binding sites," (e.g., fragments, subsequences, complementarity determining regions (CDRs)) that retain capacity to bind antigen, including (i) a Fab fragment, a monovalent

fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544-546), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR). Single chain antibodies are also included by reference in the term "antibody." Antibodies of the invention include, but are not limited to polyclonal, monoclonal, bispecific, humanized or chimeric antibodies, single chain antibodies, Fab fragments and $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above. The immunoglobulin molecules of the invention can be of any class (e.g., IgG, IgE, IgM, IgD and IgA such as IgG) or subclass of immunoglobulin molecule.

[0164] The term "specifically binds" (or "immunospecifically binds") is not intended to indicate that an antibody binds exclusively to its intended target. Rather, an antibody "specifically binds" if its affinity for its intended target is about 5-fold greater when compared to its affinity for a non-target molecule. Preferably the affinity of the antibody will be at least about 5 fold, preferably 10 fold, more preferably 25-fold, even more preferably 50-fold, and most preferably 100-fold or more, greater for a target molecule than its affinity for a non-target molecule. In some embodiments, specific binding between an antibody or other binding agent and an antigen means a binding affinity of at least $10^6 M^{-1}$. Antibodies may, for example bind with affinities of at least about $10^7 M^{-1}$, such as between about $10^8 M^{-1}$ to about $10^9 M^{-1}$, about $10^9 M^{-1}$ to about $10^{10} M^{-1}$, or about $10^{10} M^{-1}$ to about $10^{11} M^{-1}$.

[0165] Affinity is calculated as $K_d = k_{off}/k_{on}$ (k_{off} is the dissociation rate constant, k_{on} is the association rate constant and K_d is the equilibrium constant. Affinity can be determined at equilibrium by measuring the fraction bound (r) of labeled ligand at various concentrations (c). The data are graphed using the Scatchard equation:

$$r/c = K(n-r)$$

[0166] where

[0167] r =moles of bound ligand/mole of receptor at equilibrium;

[0168] c =free ligand concentration at equilibrium;

[0169] K =equilibrium association constant; and

[0170] n =number of ligand binding sites per receptor molecule

By graphical analysis, r/c is plotted on the Y-axis versus r on the X-axis thus producing a Scatchard plot. The affinity is the negative slope of the line. k_{off} can be determined by competing bound labeled ligand with unlabeled excess ligand (see, e.g., U.S. Pat. No. 6,316,409). The affinity of a targeting agent for its target molecule is for example at least about 1×10^{-6} moles/liter, such as at least about 1×10^{-7} moles/liter, is such as at least about 1×10^{-8} moles/liter, especially at least about 1×10^{-9} moles/liter, and particularly at least about 1×10^{-10} moles/liter. Antibody affinity measurement by Scatchard analysis is well known in the art. See, e.g., van Erp et al., *J. Immunoassay* 12: 425-43, 1991; Nelson and Griswold, *Comput. Methods Programs Biomed.* 27: 65-8, 1988.

[0171] In one embodiment, antibodies that recognize gene products of genes encoding OGTA066 are publicly available.

In another embodiment, methods known to those skilled in the art are used to produce antibodies that recognize OGTA066, an OGTA066 analog, an OGTA066-related polypeptide, or a fragment or derivative of any of the foregoing. One skilled in the art will recognize that many procedures are available for the production of antibodies, for example, as described in *Antibodies, A Laboratory Manual*, Ed Harlow and David Lane, Cold Spring Harbor Laboratory (1988), Cold Spring Harbor, N.Y. One skilled in the art will also appreciate that binding fragments or Fab fragments which mimic antibodies can also be prepared from genetic information by various procedures (*Antibody Engineering: A Practical Approach* (Borrebaeck, C., ed.), 1995, Oxford University Press, Oxford; *J. Immunol.* 149, 3914-3920 (1992)).

[0172] In one embodiment of the invention, antibodies to a specific domain of OGTA066 are produced. In a specific embodiment, hydrophilic fragments of OGTA066 are used as immunogens for antibody production.

[0173] In the production of antibodies, screening for the desired antibody can be accomplished by techniques known in the art, e.g. ELISA (enzyme-linked immunosorbent assay). For example, to select antibodies which recognize a specific domain of OGTA066, one may assay generated hybridomas for a product which binds to an OGTA066 fragment containing such domain. For selection of an antibody that specifically binds a first OGTA066 homolog but which does not specifically bind to (or binds less avidly to) a second OGTA066 homolog, one can select on the basis of positive binding to the first OGTA066 homolog and a lack of binding to (or reduced binding to) the second OGTA066 homolog. Similarly, for selection of an antibody that specifically binds OGTA066 but which does not specifically bind to (or binds less avidly to) a different isoform of the same protein (such as a different glycoform having the same core peptide as OGTA066, one can select on the basis of positive binding to OGTA066 and a lack of binding to (or reduced binding to) the different isoform (e.g., a different glycoform). Thus, the present invention provides an antibody (such as a monoclonal antibody) that binds with greater affinity (for example at least 2-fold, more such as at least 5-fold, particularly at least 10-fold greater affinity) to OGTA066 than to a different isoform or isoforms (e.g., glycoforms) of OGTA066.

[0174] Polyclonal antibodies which may be used in the methods of the invention are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Unfractionated immune serum can also be used. Various procedures known in the art may be used for the production of polyclonal antibodies to OGTA066 a fragment of OGTA066, an OGTA066-related polypeptide, or a fragment of an OGTA066-related polypeptide. For example, one way is to purify polypeptides of interest or to synthesize the polypeptides of interest using, e.g., solid phase peptide synthesis methods well known in the art. See, e.g., *Guide to Protein Purification*, Murray P. Deutcher, ed., *Meth. Enzymol.* Vol 182 (1990); *Solid Phase Peptide Synthesis*, Greg B. Fields ed., *Meth. Enzymol.* Vol 289 (1997); Kiso et al., *Chem. Pharm. Bull.* (Tokyo) 38: 1192-99, 1990; Mostafavi et al., *Biomed. Pept. Proteins Nucleic Acids* 1: 255-60, 1995; Fujiwara et al., *Chem. Pharm. Bull.* (Tokyo) 44: 1326-31, 1996. The selected polypeptides may then be used to immunize by injection various host animals, including but not limited to rabbits, mice, rats, etc., to generate polyclonal or monoclonal antibodies. The Preferred Technology described herein in Example 1 provides isolated OGTA066 suitable for such

immunization. If OGTA066 is purified by gel electrophoresis, OGTA066 can be used for immunization with or without prior extraction from the polyacrylamide gel. Various adjuvants (i.e. immunostimulants) may be used to enhance the immunological response, depending on the host species, including, but not limited to, complete or incomplete Freund's adjuvant, a mineral gel such as aluminum hydroxide, surface active substance such as lysolecithin, pluronic polyol, a polyanion, a peptide, an oil emulsion, keyhole limpet hemocyanin, dinitrophenol, and an adjuvant such as BCG (*bacille Calmette-Guerin*) or *corynebacterium parvum*. Additional adjuvants are also well known in the art.

[0175] For preparation of monoclonal antibodies (mAbs) directed toward OGTA066 a fragment of OGTA066, an OGTA066-related polypeptide, or a fragment of an OGTA066-related polypeptide, any technique which provides for the production of antibody molecules by continuous cell lines in culture may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (1975, *Nature* 256:495-497), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., 1985, in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, IgD and any subclass thereof. The hybridoma producing the mAbs of the invention may be cultivated in vitro or in vivo. In an additional embodiment of the invention, monoclonal antibodies can be produced in germ-free animals utilizing known technology (PCT/US90/02545, incorporated herein by reference).

[0176] The monoclonal antibodies include but are not limited to human monoclonal antibodies and chimeric monoclonal antibodies (e.g., human-mouse chimeras). A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a human immunoglobulin constant region and a variable region derived from a murine mAb. (See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; and Boss et al., U.S. Pat. No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Pat. No. 5,585,089, which is incorporated herein by reference in its entirety.)

[0177] Chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods described in PCT Publication No. WO 87/02671; European Patent Application 184,187; European Patent Application 171,496; European Patent Application 173,494; PCT Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Application 125,023; Better et al., 1988, *Science* 240:1041-1043; Liu et al., 1987, *Proc. Natl. Acad. Sci. USA* 84:3439-3443; Liu et al., 1987, *J. Immunol.* 139:3521-3526; Sun et al., 1987, *Proc. Natl. Acad. Sci. USA* 84:214-218; Nishimura et al., 1987, *Canc. Res.* 47:999-1005; Wood et al., 1985, *Nature* 314:446-449; and Shaw et al., 1988, *J. Natl. Cancer Inst.* 80:1553-1559; Morrison, 1985, *Science* 229:1202-1207; Oi et al., 1986, *Bio/Techniques* 4:214; U.S. Pat. No. 5,225,539; Jones et al., 1986, *Nature* 321:552-525; Verhoeyan et al. (1988) *Science* 239:1534; and Beidler et al., 1988, *J. Immunol.* 141:4053-4060.

[0178] Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Such antibodies can be produced using transgenic mice which are incapable of expressing endogenous immunoglobulin heavy and light chain genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of OGTA066. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., U.S. Pat. No. 5,625,126; U.S. Pat. No. 5,633,425; U.S. Pat. No. 5,569,825; U.S. Pat. No. 5,661,016; and U.S. Pat. No. 5,545,806. In addition, companies such as Abgenix, Inc. (Freemont, Calif.) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0179] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al. (1994) *Bio/technology* 12:899-903).

[0180] The antibodies of the present invention can also be generated by the use of phage display technology to produce and screen libraries of polypeptides for binding to a selected target. See, e.g., Cwirla et al., *Proc. Natl. Acad. Sci. USA* 87, 6378-82, 1990; Devlin et al., *Science* 249, 404-6, 1990; Scott and Smith, *Science* 249, 386-88, 1990; and Ladner et al., U.S. Pat. No. 5,571,698. A basic concept of phage display methods is the establishment of a physical association between DNA encoding a polypeptide to be screened and the polypeptide. This physical association is provided by the phage particle, which displays a polypeptide as part of a capsid enclosing the phage genome which encodes the polypeptide. The establishment of a physical association between polypeptides and their genetic material allows simultaneous mass screening of very large numbers of phage bearing different polypeptides. Phage displaying a polypeptide with affinity to a target bind to the target and these phage are enriched by affinity screening to the target. The identity of polypeptides displayed from these phage can be determined from their respective genomes. Using these methods a polypeptide identified as having a binding affinity for a desired target can then be synthesized in bulk by conventional means. See, e.g., U.S. Pat. No. 6,057,098, which is hereby incorporated in its entirety, including all tables, figures, and claims. In particular, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding

domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); PCT Application No. PCT/GB91/01134; PCT Publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0181] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[0182] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988).

[0183] The invention further provides for the use of bispecific antibodies, which can be made by methods known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Trauneker et al., 1991, *EMBO J.* 10:3655-3659.

[0184] According to a different and more preferred approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host

organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0185] In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690 published Mar. 3, 1994. For further details for generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 1986, 121:210.

[0186] The invention provides functionally active fragments, derivatives or analogs of anti-OGTA066 immunoglobulin molecules. Functionally active means that the fragment, derivative or analog is able to elicit anti-anti-idiotypic antibodies (i.e. tertiary antibodies) that recognize the same antigen that is recognized by the antibody from which the fragment, derivative or analog is derived. Specifically, in a preferred embodiment the antigenicity of the idiotype of the immunoglobulin molecule may be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art.

[0187] The present invention provides antibody fragments such as, but not limited to, F(ab')₂ fragments and Fab fragments. Antibody fragments which recognize specific epitopes may be generated by known techniques. F(ab')₂ fragments consist of the variable region, the light chain constant region and the CH1 domain of the heavy chain and are generated by pepsin digestion of the antibody molecule. Fab fragments are generated by reducing the disulfide bridges of the F(ab')₂ fragments. The invention also provides heavy chain and light chain dimers of the antibodies of the invention, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (e.g., as described in U.S. Pat. No. 4,946,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the antibody of the invention. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may be used (Skerra et al., 1988, *Science* 242:1038-1041).

[0188] In other embodiments, the invention provides fusion proteins of the immunoglobulins of the invention (or functionally active fragments thereof), for example in which the immunoglobulin is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino

acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the immunoglobulin. Preferably the immunoglobulin, or fragment thereof, is covalently linked to the other protein at the N-terminus of the constant domain. As stated above, such fusion proteins may facilitate purification, increase half-life in vivo, and enhance the delivery of an antigen across an epithelial barrier to the immune system.

[0189] The immunoglobulins of the invention include analogs and derivatives that are modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment does not impair immunospecific binding. For example, but not by way of limitation, the derivatives and analogs of the immunoglobulins include those that have been further modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, etc. Additionally, the analog or derivative may contain one or more non-classical amino acids.

[0190] The foregoing antibodies can be used in methods known in the art relating to the localization and activity of OGTA066, e.g. for imaging this protein, measuring levels thereof in appropriate physiological samples, in diagnostic methods, etc.

Production of Affibodies to OGTA066

[0191] Affibody molecules represent a new class of affinity proteins based on a 58-amino acid residue protein domain, derived from one of the IgG-binding domains of staphylococcal protein A. This three helix bundle domain has been used as a scaffold for the construction of combinatorial phagemid libraries, from which Affibody variants that target the desired molecules can be selected using phage display technology (Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren P A, Binding proteins selected from combinatorial libraries of an α -helical bacterial receptor domain, *Nat Biotechnol* 1997; 15:772-7. Ronmark J, Gronlund H, Uhlen M, Nygren P A, Human immunoglobulin A (IgA)-specific ligands from combinatorial engineering of protein A, *Eur J Biochem* 2002; 269:2647-55.). The simple, robust structure of Affibody molecules in combination with their low molecular weight (6 kDa), make them suitable for a wide variety of applications, for instance, as detection reagents (Ronmark J, Hansson M, Nguyen T, et al, Construction and characterization of affibody-Fc chimeras produced in *Escherichia coli*, *J Immunol Methods* 2002; 261:199-211) and to inhibit receptor interactions (Sandstorm K, Xu Z, Forsberg G, Nygren P A, Inhibition of the CD28-CD80 co-stimulation signal by a CD28-binding Affibody ligand developed by combinatorial protein engineering, *Protein Eng* 2003; 16:691-7). Further details of Affibodies and methods of production thereof may be obtained by reference to U.S. Pat. No. 5,831,012 which is herein incorporated by reference in its entirety.

[0192] Labelled Affibodies may also be useful in imaging applications for determining abundance of Isoforms.

Production of Domain Antibodies to OGTA066

[0193] References to antibodies herein embrace references to Domain Antibodies. Domain Antibodies (dAbs) are the

smallest functional binding units of antibodies, corresponding to the variable regions of either the heavy (V_H) or light (V_L) chains of human antibodies. Domain Antibodies have a molecular weight of approximately 13 kDa. Domantis has developed a series of large and highly functional libraries of fully human V_H and V_L dAbs (more than ten billion different sequences in each library), and uses these libraries to select dAbs that are specific to therapeutic targets. In contrast to many conventional antibodies, Domain Antibodies are well expressed in bacterial, yeast, and mammalian cell systems. Further details of domain antibodies and methods of production thereof may be obtained by reference to U.S. Pat. Nos. 6,291,158; 6,582,915; 6,593,081; 6,172,197; 6,696,245; US Serial No. 2004/0110941; European patent application No. 1433846 and European Patents 0368684 & 0616640; WO05/035572, WO04/101790, WO04/081026, WO04/058821, WO04/003019 and WO03/002609, each of which is herein incorporated by reference in its entirety.

Production of Nanobodies to OGTA066

[0194] Nanobodies are antibody-derived therapeutic proteins that contain the unique structural and functional properties of naturally-occurring heavy-chain antibodies. These heavy-chain antibodies contain a single variable domain (V_{HH}) and two constant domains (C_{H2} and C_{H3}). Importantly, the cloned and isolated domain is a perfectly stable polypeptide harbouring the full antigen-binding capacity of the original heavy-chain antibody. Nanobodies have a high homology with the V_H domains of human antibodies and can be further humanised without any loss of activity. Importantly, Nanobodies have a low immunogenic potential, which has been confirmed in primate studies with Nanobody lead compounds.

[0195] Nanobodies combine the advantages of conventional antibodies with important features of small molecule drugs. Like conventional antibodies, Nanobodies show high target specificity, high affinity for their target and low inherent toxicity. However, like small molecule drugs they can inhibit enzymes and readily access receptor clefts. Furthermore, Nanobodies are extremely stable, can be administered by means other than injection (see e.g. WO 04/041867, which is herein incorporated by reference in its entirety) and are easy to manufacture. Other advantages of Nanobodies include recognising uncommon or hidden epitopes as a result of their small size, binding into cavities or active sites of protein targets with high affinity and selectivity due to their unique 3-dimensional, drug format flexibility, tailoring of half-life and ease and speed of drug discovery.

[0196] Nanobodies are encoded by single genes and are efficiently produced in almost all prokaryotic and eukaryotic hosts e.g. *E. coli* (see e.g. U.S. Pat. No. 6,765,087, which is herein incorporated by reference in its entirety), moulds (for example *Aspergillus* or *Trichoderma*) and yeast (for example *Saccharomyces*, *Kluyveromyces*, *Hansenula* or *Pichia*) (see e.g. U.S. Pat. No. 6,838,254, which is herein incorporated by reference in its entirety). The production process is scalable and multi-kilogram quantities of Nanobodies have been produced. Because Nanobodies exhibit a superior stability compared with conventional antibodies, they can be formulated as a long shelf-life, ready-to-use solution.

[0197] The Nanoclone method (see e.g. WO 06/079372, which is herein incorporated by reference in its entirety) is a

proprietary method for generating Nanobodies against a desired target, based on automated high-throughout selection of B-cells.

Production of UniBodies to OGTA066

[0198] UniBody is a new proprietary antibody technology that creates a stable, smaller antibody format with an anticipated longer therapeutic window than current small antibody formats. IgG4 antibodies are considered inert and thus do not interact with the immune system. Genmab modified fully human IgG4 antibodies by eliminating the hinge region of the antibody. Unlike the full size IgG4 antibody, the half molecule fragment is very stable and is termed a UniBody. Halving the IgG4 molecule left only one area on the UniBody that can bind to disease targets and the UniBody therefore binds univalently to only one site on target cells. This univalent binding does not stimulate cancer cells to grow like bivalent antibodies might and opens the door for treatment of some types of cancer which ordinary antibodies cannot treat.

[0199] The UniBody is about half the size of a regular IgG4 antibody. This small size can be a great benefit when treating some forms of cancer, allowing for better distribution of the molecule over larger solid tumors and potentially increasing efficacy.

[0200] Fabs typically do not have a very long half-life. UniBodies, however, were cleared at a similar rate to whole IgG4 antibodies and were able to bind as well as whole antibodies and antibody fragments in pre-clinical studies. Other antibodies primarily work by killing the targeted cells whereas UniBodies only inhibit or silence the cells.

[0201] Further details of UniBodies may be obtained by reference to patent WO2007/059782, which is herein incorporated by reference in its entirety.

Expression of Affinity Reagents

Expression of Antibodies

[0202] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

[0203] Recombinant expression of antibodies, or fragments, derivatives or analogs thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *Bio Techniques* 17:242), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0204] Alternatively, the nucleic acid encoding the antibody may be obtained by cloning the antibody. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

[0205] If an antibody molecule that specifically recognizes a particular antigen is not available (or a source for a cDNA library for cloning a nucleic acid encoding such an antibody), antibodies specific for a particular antigen may be generated by any method known in the art, for example, by immunizing an animal, such as a rabbit, to generate polyclonal antibodies or, for example, by generating monoclonal antibodies. Alternatively, a clone encoding at least the Fab portion of the antibody may be obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

[0206] Once a nucleic acid encoding at least the variable domain of the antibody molecule is obtained, it may be introduced into a vector containing the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464). Vectors containing the complete light or heavy chain for co-expression with the nucleic acid to allow the expression of a complete antibody molecule are also available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitution(s) or deletion(s) necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulfhydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis, in vitro site directed mutagenesis (Hutchinson et al., 1978, *J. Biol. Chem.* 253:6551), PCT based methods, etc.

[0207] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human antibody constant region, e.g., humanized antibodies.

[0208] Once a nucleic acid encoding an antibody molecule of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing the proteins of the invention by expressing nucleic acid containing the antibody molecule sequences are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody molecule coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) and Ausubel et al. (eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY).

[0209] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention.

[0210] The host cells used to express a recombinant antibody of the invention may be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus are an effective expression system for antibodies (Foecking et al., 1986, Gene 45:101; Cockett et al., 1990, Bio/Technology 8:2).

[0211] A variety of host-expression vector systems may be utilized to express an antibody molecule of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0212] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions comprising an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, EMBO J. 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0213] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter). In mammalian host cells, a number of viral-based expression systems (e.g., an adenovirus expression system) may be utilized.

[0214] As discussed above, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein.

[0215] For long-term, high-yield production of recombinant antibodies, stable expression is preferred. For example, cell lines that stably express an antibody of interest can be produced by transfecting the cells with an expression vector comprising the nucleotide sequence of the antibody and the nucleotide sequence of a selectable (e.g., neomycin or hygromycin), and selecting for expression of the selectable marker. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[0216] The expression levels of the antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

[0217] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0218] Once an antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an antibody molecule, for example, by chromatography (e.g., ion exchange chromatography, affinity chromatography such as with protein A or specific antigen, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

[0219] Alternatively, any fusion protein may be readily purified by utilizing an antibody specific for the fusion protein being expressed. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia

recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with recombinant vaccinia virus are loaded onto Ni²⁺ nitriloacetic acid-agarose columns and histidine-tagged proteins are selectively eluted with imidazole-containing buffers. [0220] The antibodies that are generated by these methods may then be selected by first screening for affinity and specificity with the purified polypeptide of interest and, if required, comparing the results to the affinity and specificity of the antibodies with polypeptides that are desired to be excluded from binding. The screening procedure can involve immobilization of the purified polypeptides in separate wells of microtiter plates. The solution containing a potential antibody or groups of antibodies is then placed into the respective microtiter wells and incubated for about 30 min to 2 h. The microtiter wells are then washed and a labeled secondary antibody (for example, an anti-mouse antibody conjugated to alkaline phosphatase if the raised antibodies are mouse antibodies) is added to the wells and incubated for about 30 min and then washed. Substrate is added to the wells and a color reaction will appear where antibody to the immobilized polypeptide(s) is present.

[0221] The antibodies so identified may then be further analyzed for affinity and specificity in the assay design selected. In the development of immunoassays for a target protein, the purified target protein acts as a standard with which to judge the sensitivity and specificity of the immunoassay using the antibodies that have been selected. Because the binding affinity of various antibodies may differ; certain antibody pairs (e.g., in sandwich assays) may interfere with one another sterically, etc., assay performance of an antibody may be a more important measure than absolute affinity and specificity of an antibody.

[0222] Those skilled in the art will recognize that many approaches can be taken in producing antibodies or binding fragments and screening and selecting for affinity and specificity for the various polypeptides, but these approaches do not change the scope of the invention.

[0223] For therapeutic applications, antibodies (particularly monoclonal antibodies) may suitably be human or humanized animal (e.g. mouse) antibodies. Animal antibodies may be raised in animals using the human protein (e.g. OGTA066) as immunogen. Humanisation typically involves grafting CDRs identified thereby into human framework regions. Normally some subsequent retromutation to optimize the conformation of chains is required. Such processes are known to persons skilled in the art.

[0224] In one embodiment of the invention the antibodies or affinity reagent or part thereof employed specifically bind a sequence as disclosed in Sequence ID Nos 1, 2, 3, 4 or 5 as described herein.

Expression of Affibodies

[0225] The construction of affibodies has been described elsewhere (Ronmark J, Gronlund H, Uhlen, M., Nygren P. A, Human immunoglobulin A (IgA)-specific ligands from combinatorial engineering of protein A, 2002, Eur. J. Biochem. 269, 2647-2655.), including the construction of affibody phage display libraries (Nord, K., Nilsson, J., Nilsson, B., Uhlen, M. & Nygren, P. A, A combinatorial library of an a-helical bacterial receptor domain, 1995, Protein Eng. 8, 601-608. Nord, K., Gunneriusson, E., Ringdahl, J., Stahl, S.,

Uhlen, M. & Nygren, P. A, Binding proteins selected from combinatorial libraries of an a-helical bacterial receptor domain, 1997, Nat. Biotechnol. 15, 772-777.)

[0226] The biosensor analyses to investigate the optimal affibody variants using biosensor binding studies has also been described elsewhere (Ronmark J, Gronlund H, Uhlen, M., Nygren P. A, Human immunoglobulin A (IgA)-specific ligands from combinatorial engineering of protein A, 2002, Eur. J. Biochem. 269, 2647-2655.).

Affinity Reagent Modifications

[0227] In a preferred embodiment, anti-OGTA066 affinity reagents such as antibodies or fragments thereof are conjugated to a diagnostic moiety (such as a detectable label) or a therapeutic moiety. The antibodies can be used for diagnosis or to determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance (label). Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive nuclides, positron emitting metals (for use in positron emission tomography), and nonradioactive paramagnetic metal ions. See generally U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; suitable prosthetic groups include streptavidin, avidin and biotin; suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride and phycoerythrin; suitable luminescent materials include luminol; suitable bioluminescent materials include luciferase, luciferin, and aequorin; and suitable radioactive nuclides include ¹²⁵I, ¹³¹I, ¹¹¹In and ⁹⁹Tc. ⁶⁸Ga may also be employed.

[0228] Anti-OGTA066 antibodies or fragments thereof as well as other affinity reagents can be conjugated to a therapeutic agent or drug moiety to modify a given biological response. An exemplary therapeutic agent to which the affinity reagent may be conjugated is a cytotoxic moiety. The therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumour necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, a biological response modifier such as a lymphokine, interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), nerve growth factor (NGF) or other growth factor.

[0229] Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Appli-

cations, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.*, 62:119-58 (1982).

[0230] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Pat. No. 4,676,980.

[0231] An antibody with or without a therapeutic moiety conjugated to it can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

[0232] The invention also provides for fully human, or humanised antibodies that induce antibody-directed cell-mediated cytotoxicity (ADCC). A fully human antibody is one in which the protein sequences are encoded by naturally occurring human immunoglobulin sequences, either from isolated antibody-producing human B-lymphocytes, or from transgenic murine B-lymphocytes of mice in which the murine immunoglobulin coding chromosomal regions have been replaced by orthologous human sequences. Transgenic antibodies of the latter type include, but are not restricted to, HuMab (Medarex, Inc, CA) and Xenomouse (Abgenix Inc., CA). A humanised antibody is one in which the constant region of a non-human antibody molecule of appropriate antigen specificity, is replaced by the constant region of a human antibody, preferably of the IgG subtype, with appropriate effector functions (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454). Appropriate effector functions include ADCC, which is a natural process by which fully-human antibodies or humanized antibodies, when bound to targets on the surface of cancer cells, switch on the cell killing properties of lymphocytes that are part of the normal immune system. These active lymphocytes, called Natural Killer (NK) cells, use a cytotoxic process to destroy living cells to which the antibodies are bound. ADCC activity may be detected and quantified by measuring release of Europium (Eu3+) from Eu3+ labelled, living cells in the presence of an antigen-specific antibody and peripheral blood mononuclear cells extracted from an immunocompetent, living human subject. The ADCC process is described in detail in Janeway Jr. C. A. et al., *Immunobiology*, 5th ed., 2001, Garland Publishing, ISBN 0-8153-3642-X; Pier G. B. et al., *Immunology, Infection, and Immunity*, 2004, p 246-5; Albanell J. et al., *Advances in Experimental Medicine and Biology*, 2003, 532:p 2153-68 and Weng, W.-K. et al., *Journal of Clinical Oncology*, 2003, 21:p 3940-3947. Suitable methods for the detection and quantification of ADCC can be found in Blomberg et al., *Journal of Immunological Methods*. 1986, 86:p 225-9; Blomberg et al., *Journal of Immunological Methods*. 1986, 21; 92:p 117-23 and Patel & Boyd, *Journal of Immunological Methods*. 1995, 184:p 29-38.

[0233] ADCC typically involves activation of NK cells and is dependent on the recognition of antibody-coated cells by Fc receptors on the surface of the NK cell. The Fc receptors recognize the Fc (crystalline) portion of antibodies such as IgG, bound specifically to the surface of a target cell. The Fc receptor that triggers activation of the NK cell is called CD16 or FcγRIIIa. Once the FcγRIIIa receptor is bound to the IgG Fc, the NK cell releases cytokines such as IFN-γ, and cyto-

toxic granules containing perforin and granzymes that enter the target cell and promote cell death by triggering apoptosis.

[0234] The induction of antibody-dependent cellular cytotoxicity (ADCC) by an antibody can be enhanced by modifications that alter interactions between the antibody constant region (Fc) and various receptors that are present on the surface of cells of the immune system. Such modifications include the reduction or absence of alpha-1,6-linked fucose moieties in the complex oligosaccharide chains that are normally added to the Fc of antibodies during natural or recombinant synthesis in mammalian cells. In a preferred embodiment, non-fucosylated anti-OGTA066 affinity reagents such as antibodies or fragments thereof are produced for the purpose of enhancing their ability to induce the ADCC response.

[0235] Techniques for reducing or ablating alpha 1,6-linked fucose moieties in the oligosaccharide chains of the Fc are well established. In one example, the recombinant antibody is synthesized in a cell line that is impaired in its ability to add fucose in an alpha 1,6 linkage to the innermost N-acetylglucosamine of the N-linked biantennary complex-type Fc oligosaccharides. Such cell lines include, but are not limited to, the rat hybridoma YB2/0, which expresses a reduced level of the alpha 1,6-fucosyltransferase gene, FUT8. Preferably, the antibody is synthesized in a cell line that is incapable of adding alpha 1,6-linked fucosyl moieties to complex oligosaccharide chains, due to the deletion of both copies of the FUT8 gene. Such cell lines include, but are not limited to, FUT8-/- CHO/DG44 cell lines. Techniques for synthesizing partially fucosylated, or non-fucosylated antibodies and affinity reagents are described in Shinkawa et al., *J. Biol. Chem.* 278:3466-34735 (2003); Yamane-Ohnuki et al., *Biotechnology and Bioengineering* 87: 614-22 (2004) and in WO00/61739 A1, WO02/31140 A1 and WO03/085107 A1. In a second example, the fucosylation of a recombinant antibody is reduced or abolished by synthesis in a cell line that has been genetically engineered to overexpress a glycoprotein-modifying glycosyl transferase at a level that maximizes the production of complex N-linked oligosaccharides carrying bisecting N-acetylglucosamine. For example, the antibody is synthesized in a Chinese Hamster Ovary cell line expressing the enzyme N-acetyl glucosamine transferase III (GnT III). Cell lines stably transfected with suitable glycoprotein-modifying glycosyl transferases, and methods of synthesizing antibodies using these cells are described in WO9954342.

[0236] A non-fucosylated antibody or affinity reagent can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

[0237] In a further modification, the amino acid sequences of the antibody Fc are altered in a way that enhances ADCC activation, without affecting ligand affinity. Examples of such modifications are described in Lazar et al., *Proceedings of the National Academy of Sciences* 2006, 103:p 4005-4010; WO03074679 and WO2007039818. In these examples, substitution of amino acids in the antibody Fc, such as aspartate for serine at position 239, and isoleucine for glutamate at position 332, altered the binding affinity of an antibody for Fc receptors, leading to an increase in ADCC activation.

[0238] An antibody reagent with enhanced ADCC activation due to amino acid substitutions can be used as a therapeutic that is administered alone or in combination with cytotoxic factor(s) and/or cytokine(s).

Diagnosis of Colorectal Cancer, Kidney Cancer, Lung Cancer or Pancreatic Cancer

[0239] In accordance with the present invention, test samples of colorectal, kidney, lung or pancreatic tissue,

serum, plasma or urine obtained from a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer can be used for diagnosis or monitoring. In one embodiment, a change in the abundance of OGTA066 in a test sample relative to a control sample (from a subject or subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer) or a previously determined reference range indicates the presence of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. In another embodiment, the relative abundance of OGTA066 in a test sample compared to a control sample or a previously determined reference range indicates a subtype of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer (e.g. familial or sporadic colorectal cancer, squamous cell lung carcinoma or endocrine tumours of the pancreas). In yet another embodiment, the relative abundance of OGTA066 in a test sample relative to a control sample or a previously determined reference range indicates the degree or severity of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer (e.g. the likelihood for metastasis). In any of the aforesaid methods, detection of OGTA066 may optionally be combined with detection of one or more additional biomarkers for colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Any suitable method in the art can be employed to measure the level of OGTA066, including but not limited to the Preferred Technologies described herein in Examples 1 and 2, kinase assays, immunoassays to detect and/or visualize the OGTA066 (e.g. Western blot, immunoprecipitation followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc.). In a further embodiment, a change in the abundance of mRNA encoding OGTA066 in a test sample relative to a control sample or a previously determined reference range indicates the presence of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Any suitable hybridization assay can be used to detect OGTA066 expression by detecting and/or visualizing mRNA encoding OGTA066 (e.g. Northern assays, dot blots, in situ hybridization, etc.).

[0240] In another embodiment of the invention, labeled antibodies (or other affinity reagents), derivatives and analogs thereof, which specifically bind to OGTA066 can be used for diagnostic purposes to detect, diagnose, or monitor colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Preferably, colorectal cancer, kidney cancer, lung cancer or pancreatic cancer is detected in an animal, for example in a mammal such as a human.

Screening Assays

[0241] The invention provides methods for identifying agents (e.g. candidate compounds or test compounds) that bind to OGTA066 or have a stimulatory or inhibitory effect on the expression or activity of OGTA066. The invention also provides methods of identifying agents, candidate compounds or test compounds that bind to an OGTA066-related polypeptide or an OGTA066 fusion protein or have a stimulatory or inhibitory effect on the expression or activity of an OGTA066-related polypeptide or an OGTA066 fusion protein. Examples of agents, candidate compounds or test compounds include, but are not limited to, nucleic acids (e.g. DNA and RNA), carbohydrates, lipids, proteins, peptides, peptidomimetics, small molecules and other drugs. Agents can be obtained using any of the numerous approaches in combinatorial library methods known in the art, including: biological libraries; spatially addressable parallel solid phase

or solution phase libraries; synthetic library methods requiring deconvolution; the "one-bead one-compound" library method; and synthetic library methods using affinity chromatography selection. The biological library approach is limited to peptide libraries, while the other four approaches are applicable to peptide, non-peptide oligomer or small molecule libraries of compounds (Lam, 1997, *Anticancer Drug Des.* 12:145; U.S. Pat. No. 5,738,996; and U.S. Pat. No. 5,807,683, each of which is incorporated herein in its entirety by reference).

[0242] Examples of methods for the synthesis of molecular libraries can be found in the art, for example in: DeWitt et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:6909; Erb et al., 1994, *Proc. Natl. Acad. Sci. USA* 91:11422; Zuckermann et al., 1994, *J. Med. Chem.* 37:2678; Cho et al., 1993, *Science* 261:1303; Carrell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2059; Carell et al., 1994, *Angew. Chem. Int. Ed. Engl.* 33:2061; and Gallop et al., 1994, *J. Med. Chem.* 37:1233, each of which is incorporated herein in its entirety by reference.

[0243] Libraries of compounds may be presented, e.g. presented in solution (e.g. Houghten, 1992, *Bio/Techniques* 13:412-421), or on beads (Lam, 1991, *Nature* 354:82-84), chips (Fodor, 1993, *Nature* 364:555-556), bacteria (U.S. Pat. No. 5,223,409), spores (U.S. Pat. Nos. 5,571,698; 5,403,484; and 5,223,409), plasmids (Cull et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:1865-1869) or phage (Scott and Smith, 1990, *Science* 249:386-390; Devlin, 1990, *Science* 249:404-406; Cwirla et al., 1990, *Proc. Natl. Acad. Sci. USA* 87:6378-6382; and Felici, 1991, *J. Mol. Biol.* 222:301-310), each of which is incorporated herein in its entirety by reference.

[0244] In one embodiment, agents that interact with (i.e. bind to) OGTA066, an OGTA066 fragment (e.g. a functionally active fragment), an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein are identified in a cell-based assay system. In accordance with this embodiment, cells expressing OGTA066, a fragment of OGTA066, an OGTA066-related polypeptide, a fragment of the OGTA066-related polypeptide, or an OGTA066 fusion protein are contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with the OGTA066 is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate compounds. The cell, for example, can be of prokaryotic origin (e.g. *E. coli*) or eukaryotic origin (e.g. yeast or mammalian). Further, the cells can express OGTA066, a fragment of OGTA066, an OGTA066-related polypeptide, a fragment of the OGTA066-related polypeptide, or an OGTA066 fusion protein endogenously or be genetically engineered to express OGTA066, a fragment of OGTA066, an OGTA066-related polypeptide, a fragment of the OGTA066-related polypeptide, or an OGTA066 fusion protein. In certain instances, OGTA066, a fragment of OGTA066, an OGTA066-related polypeptide, a fragment of the OGTA066-related polypeptide, or an OGTA066 fusion protein or the candidate compound is labeled, for example with a radioactive label (such as ³²P, ³⁵S, and ¹²⁵I) or a fluorescent label (such as fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde or fluorescamine) to enable detection of an interaction between OGTA066 and a candidate compound. The ability of the candidate compound to interact directly or indirectly with OGTA066, a fragment of an OGTA066, an OGTA066-related polypeptide, a fragment of an OGTA066-

related polypeptide, or an OGTA066 fusion protein can be determined by methods known to those of skill in the art. For example, the interaction between a candidate compound and OGTA066, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein can be determined by flow cytometry, a scintillation assay, immunoprecipitation or western blot analysis.

[0245] In another embodiment, agents that interact with (i.e. bind to) OGTA066, an OGTA066 fragment (e.g. a functionally active fragment), an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein are identified in a cell-free assay system. In accordance with this embodiment, a native or recombinant OGTA066 or fragment thereof, or a native or recombinant OGTA066-related polypeptide or fragment thereof, or an OGTA066-fusion protein or fragment thereof, is contacted with a candidate compound or a control compound and the ability of the candidate compound to interact with OGTA066 or OGTA066-related polypeptide, or OGTA066 fusion protein is determined. If desired, this assay may be used to screen a plurality (e.g. a library) of candidate compounds. Preferably, OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein is first immobilized, by, for example, contacting OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein with an immobilized antibody (or other affinity reagent) which specifically recognizes and binds it, or by contacting a purified preparation of OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein with a surface designed to bind proteins. OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein may be a fusion protein comprising OGTA066 or a biologically active portion thereof, or OGTA066-related polypeptide and a domain such as glutathionine-S-transferase. Alternatively, OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide or an OGTA066 fusion protein can be biotinylated using techniques well known to those of skill in the art (e.g. biotinylation kit, Pierce Chemicals; Rockford, Ill.). The ability of the candidate compound to interact with OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein can be determined by methods known to those of skill in the art.

[0246] In another embodiment, a cell-based assay system is used to identify agents that bind to or modulate the activity of a protein, such as an enzyme, or a biologically active portion thereof, which is responsible for the production or degradation of OGTA066 or is responsible for the post-translational modification of OGTA066. In a primary screen, a plurality (e.g. a library) of compounds are contacted with cells that naturally or recombinantly express: (i) OGTA066, an isoform of OGTA066, an OGTA066 homolog, an OGTA066-related polypeptide, an OGTA066 fusion protein, or a biologically active fragment of any of the foregoing; and (ii) a protein that

is responsible for processing of OGTA066, an OGTA066 isoform, an OGTA066 homolog, an OGTA066-related polypeptide, an OGTA066 fusion protein, or a fragment in order to identify compounds that modulate the production, degradation, or post-translational modification of OGTA066, an OGTA066 isoform, an OGTA066 homolog, an OGTA066-related polypeptide, an OGTA066 fusion protein or fragment. If desired, compounds identified in the primary screen can then be assayed in a secondary screen against cells naturally or recombinantly expressing OGTA066. The ability of the candidate compound to modulate the production, degradation or post-translational modification of OGTA066, isoform, homolog, OGTA066-related polypeptide, or OGTA066 fusion protein can be determined by methods known to those of skill in the art, including without limitation, flow cytometry, a scintillation assay, immunoprecipitation and western blot analysis.

[0247] In another embodiment, agents that competitively interact with (i.e. bind to) OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein are identified in a competitive binding assay. In accordance with this embodiment, cells expressing OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein are contacted with a candidate compound and a compound known to interact with OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide or an OGTA066 fusion protein; the ability of the candidate compound to preferentially interact with OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein is then determined. Alternatively, agents that preferentially interact with (i.e. bind to) OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide or fragment of an OGTA066-related polypeptide are identified in a cell-free assay system by contacting OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein with a candidate compound and a compound known to interact with OGTA066, an OGTA066-related polypeptide or an OGTA066 fusion protein. As stated above, the ability of the candidate compound to interact with OGTA066, an OGTA066 fragment, an OGTA066-related polypeptide, a fragment of an OGTA066-related polypeptide, or an OGTA066 fusion protein can be determined by methods known to those of skill in the art. These assays, whether cell-based or cell-free, can be used to screen a plurality (e.g. a library) of candidate compounds.

[0248] In another embodiment, agents that modulate (i.e. upregulate or downregulate) the expression or activity of OGTA066 or an OGTA066-related polypeptide are identified by contacting cells (e.g. cells of prokaryotic origin or eukaryotic origin) expressing OGTA066 or an OGTA066-related polypeptide with a candidate compound or a control compound (e.g. phosphate buffered saline (PBS)) and determining the expression of OGTA066, OGTA066-related polypeptide, or OGTA066 fusion protein, mRNA encoding OGTA066, or mRNA encoding the OGTA066-related polypeptide. The level of expression of OGTA066, OGTA066-related polypeptide, mRNA encoding OGTA066, or mRNA encoding the OGTA066-related polypeptide in the presence of the candidate compound is compared to the level

of expression of OGTA066, OGTA066-related polypeptide, mRNA encoding OGTA066, or mRNA encoding the OGTA066-related polypeptide in the absence of the candidate compound (e.g. in the presence of a control compound). The candidate compound can then be identified as a modulator of the expression of OGTA066, or the OGTA066-related polypeptide based on this comparison. For example, when expression of OGTA066 or mRNA is significantly greater in the presence of the candidate compound than in its absence, the candidate compound is identified as a stimulator of expression of OGTA066 or mRNA. Alternatively, when expression of OGTA066 or mRNA is significantly less in the presence of the candidate compound than in its absence, the candidate compound is identified as an inhibitor of the expression of OGTA066 or mRNA. The level of expression of OGTA066 or the mRNA that encodes it can be determined by methods known to those of skill in the art. For example, mRNA expression can be assessed by Northern blot analysis or RT-PCR, and protein levels can be assessed by western blot analysis.

[0249] In another embodiment, agents that modulate the activity of OGTA066 or an OGTA066-related polypeptide are identified by contacting a preparation containing OGTA066 or OGTA066-related polypeptide or cells (e.g. prokaryotic or eukaryotic cells) expressing OGTA066 or OGTA066-related polypeptide with a test compound or a control compound and determining the ability of the test compound to modulate (e.g. stimulate or inhibit) the activity of OGTA066 or OGTA066-related polypeptide. The activity of OGTA066 or an OGTA066-related polypeptide can be assessed by detecting induction of a cellular signal transduction pathway of OGTA066 or OGTA066-related polypeptide (e.g. intracellular Ca^{2+} , diacylglycerol, IP₃, etc.), detecting catalytic or enzymatic activity of the target on a suitable substrate, detecting the induction of a reporter gene (e.g. a regulatory element that is responsive to OGTA066 or an OGTA066-related polypeptide and is operably linked to a nucleic acid encoding a detectable marker, e.g. luciferase), or detecting a cellular response, for example, cellular differentiation, or cell proliferation. Based on the present description, techniques known to those of skill in the art can be used for measuring these activities (see, e.g. U.S. Pat. No. 5,401,639, which is incorporated herein by reference). The candidate compound can then be identified as a modulator of the activity of OGTA066 or an OGTA066-related polypeptide by comparing the effects of the candidate compound to the control compound. Suitable control compounds include phosphate buffered saline (PBS) and normal saline (NS).

[0250] In another embodiment, agents that modulate (i.e. upregulate or downregulate) the expression, activity or both the expression and activity of OGTA066 or an OGTA066-related polypeptide are identified in an animal model. Examples of suitable animals include, but are not limited to, mice, rats, rabbits, monkeys, guinea pigs, dogs and cats. Preferably, the animal used represent a model of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer (e.g. xenografts of human colorectal cancer cell lines such as MDA-MB-345 in oestrogen-deprived SCID mice, Eccles et al. 1994 Cell Biophysics 24/25, 279; xenografts of renal cell cancer cell lines such as LABAZ1 in immune compromised mice, Zisman et al, Cancer Research 63, 4952-4959, Aug. 15, 2003; xenografts of non small cell lung cancer cell lines such as A549 and H460 and xenografts of small cell lung cancer cell lines such as NCI-H345 or xenografts of pancreatic can-

cer cell lines such as MIA PaCa-2 in nude mice, Marincola et al., J Surg Res 1989 December; 47(6):520-9). These can be utilized to test compounds that modulate OGTA066 levels, since the pathology exhibited in these models is similar to that of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. In accordance with this embodiment, the test compound or a control compound is administered (e.g. orally, rectally or parenterally such as intraperitoneally or intravenously) to a suitable animal and the effect on the expression, activity or both expression and activity of OGTA066 or OGTA066-related polypeptide is determined. Changes in the expression of OGTA066 or an OGTA066-related polypeptide can be assessed by the methods outlined above.

[0251] In yet another embodiment, OGTA066 or an OGTA066-related polypeptide is used as a "bait protein" in a two-hybrid assay or three hybrid assay to identify other proteins that bind to or interact with OGTA066 or an OGTA066-related polypeptide (see, e.g. U.S. Pat. No. 5,283,317; Zervos et al. (1993) Cell 72:223-232; Madura et al. (1993) J. Biol. Chem. 268:12046-12054; Bartel et al. (1993) Bio/Techniques 14:920-924; Iwabuchi et al. (1993) Oncogene 8:1693-1696; and PCT Publication No. WO 94/10300). As those skilled in the art will appreciate, such binding proteins are also likely to be involved in the propagation of signals by OGTA066 as, for example, upstream or downstream elements of a signaling pathway involving OGTA066.

[0252] This invention further provides novel agents identified by the above-described screening assays and uses thereof for treatments, for example as described herein. In addition, the invention also provides the use of an agent which interacts with, or modulates the activity of, OGTA066 in the manufacture of a medicament for the treatment of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

Therapeutic Use of OGTA066

[0253] The invention provides for treatment or prevention of various diseases and disorders by administration of a therapeutic compound. Such compounds include but are not limited to: OGTA066, OGTA066 analogs, OGTA066-related polypeptides and derivatives (including fragments) thereof; antibodies (or other affinity reagents) to the foregoing; nucleic acids encoding OGTA066, OGTA066 analogs, OGTA066-related polypeptides and fragments thereof; antisense nucleic acids to a gene encoding OGTA066 or an OGTA066-related polypeptide; and modulator (e.g., agonists and antagonists) of a gene encoding OGTA066 or an OGTA066-related polypeptide. Colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, may be treated (e.g. to ameliorate symptoms or to retard onset or progression) or prevented by administration of a therapeutic compound that reduces function or expression of OGTA066 in the serum or tissue of subjects having colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer.

[0254] In one embodiment, one or more antibodies (or other affinity reagents) each specifically binding to OGTA066, for example one or more of the sequences described herein are administered alone or in combination with one or more additional therapeutic compounds or treatments.

[0255] A biological product such as an antibody (or other affinity reagent) is allogeneic to the subject to which it is administered. In one embodiment, a human OGTA066 or a human OGTA066-related polypeptide, a nucleotide sequence encoding a human OGTA066 or a human OGTA066-related

polypeptide, or an antibody (or other affinity reagent) to a human OGTA066 or a human OGTA066-related polypeptide, is administered to a human subject for therapy (e.g. to ameliorate symptoms or to retard onset or progression) or prophylaxis.

[0256] Without being limited by theory, it is conceived that the therapeutic activity of antibodies (or other affinity reagents) which specifically bind to OGTA066 may be achieved through the phenomenon of Antibody-Dependent Cell-mediated Cytotoxicity (ADCC) (see e.g. Janeway Jr. C. A. et al., *Immunobiology*, 5th ed., 2001, Garland Publishing, ISBN 0-8153-3642-X; Pier G. B. et al., *Immunology, Infection, and Immunity*, 2004, p 246-5; Albanell J. et al., *Advances in Experimental Medicine and Biology*, 2003, 532:p 2153-68 and Weng, W.-K. et al., *Journal of Clinical Oncology*, 2003, 21:p 3940-3947).

Treatment and Prevention of Colorectal Cancer, Kidney Cancer, Lung Cancer and Pancreatic Cancer

[0257] Colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer are treated or prevented by administration to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, or to be at risk of developing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer of a compound that modulates (i.e., increases or decreases) the level or activity (i.e., function) of OGTA066 that is differentially present in the serum or tissue of subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, compared with serum or tissue of subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer. In one embodiment, colorectal cancer, kidney cancer, lung cancer or pancreatic cancer are treated or prevented by administering to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, or to be at risk of developing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, a compound that upregulates (i.e., increases) the level or activity (i.e., function) of OGTA066 for example that are decreased in the serum or tissue of subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Examples of such a compound include, but are not limited to, OGTA066 antisense oligonucleotides, ribozymes, antibodies (or other affinity reagents) directed against OGTA066, and compounds that inhibit the enzymatic activity of OGTA066. Other useful compounds e.g. OGTA066 antagonists and small molecule OGTA066 antagonists, can be identified using *in vitro* assays.

[0258] Colorectal cancer, kidney cancer, lung cancer, and/or pancreatic cancer, may also be treated or prevented by administration to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer or to be at risk of developing colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer, of a compound that downregulates the level or activity (i.e. function) of OGTA066 that is increased in the serum or tissue of subjects having colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer. Examples of such a compound include but are not limited to: OGTA066, OGTA066 fragments and OGTA066-related polypeptides; nucleic acids encoding OGTA066, an OGTA066 fragment and an OGTA066-related polypeptide (e.g. for use in gene therapy); and, for those OGTA066 or OGTA066-related polypeptides with enzymatic activity, compounds or molecules known to

modulate that enzymatic activity. Other compounds that can be used, e.g. OGTA066 agonists, can be identified using *in vitro* assays.

[0259] Whilst not wishing to be bound by theory it is thought that in patients with colorectal cancer, kidney cancer, lung cancer or pancreatic cancer that decreased levels of OGTA066 are observed in the serum whereas increased levels of the protein are observed in tissue samples derived from a biopsy of the cancer. Thus in one aspect the invention provides a method for detecting reduced levels of OGTA066 in serum from a patient with a relevant cancer and in another embodiment the invention provides a method for detecting elevated levels of OGTA066 in biopsy tissue from a patient with a relevant cancer.

[0260] In another embodiment, therapy or prophylaxis is tailored to the needs of an individual subject. Thus, in specific embodiments, compounds that promote the level or function of OGTA066 are therapeutically or prophylactically administered to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, in whom the levels or functions of OGTA066 are absent or are decreased relative to a control or normal reference range. In further embodiments, compounds that promote the level or function of OGTA066 are therapeutically or prophylactically administered to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, prostate in whom the levels or functions of OGTA066 are increased relative to a control or to a reference range. In further embodiments, compounds that decrease the level or function of OGTA066 are therapeutically or prophylactically administered to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in whom the levels or functions of OGTA066 are increased relative to a control or to a reference range.

[0261] In further embodiments, compounds that decrease the level or function of OGTA066 are therapeutically or prophylactically administered to a subject suspected of having or known to have colorectal cancer, kidney cancer, lung cancer or pancreatic cancer in whom the levels or functions of OGTA066 are decreased relative to a control or to a reference range. The change in OGTA066 function or level due to the administration of such compounds can be readily detected, e.g., by obtaining a sample (e.g., blood or urine) and assaying *in vitro* the levels or activities of OGTA066 or the levels of mRNAs encoding OGTA066 or any combination of the foregoing. Such assays can be performed before and after the administration of the compound as described herein.

[0262] The compounds of the invention include but are not limited to any compound, e.g., a small organic molecule, protein, peptide, antibody (or other affinity reagent), nucleic acid, etc. that restores the OGTA066 profile towards normal. The compounds of the invention may be given in combination with any other chemotherapy drugs.

Vaccine Therapy

[0263] Another aspect of the invention is an immunogenic composition, suitably a vaccine composition, comprising OGTA066 or an epitope containing fragment thereof, or nucleic acid encoding OGTA066 or a fragment thereof optionally together with an immunostimulant.

[0264] There is also provided a method of raising an immune response which comprises administering to a subject such compositions and a method for treating or preventing

colorectal cancer, kidney cancer, lung cancer or pancreatic cancer which comprises administering to a subject in need thereof a therapeutically effective amount of such compositions and such compositions for use in preventing or treating colorectal cancer, kidney cancer, lung cancer or pancreatic cancer or the use of the same in the manufacture of a medicament for the treatment or prevention of a relevant cancer.

[0265] Thus, OGTA066 may be useful as antigenic material, and may be used in the production of vaccines for treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer and pancreatic cancer. Such material can be “antigenic” and/or “immunogenic”.

[0266] Generally, “antigenic” is taken to mean that the protein is capable of being used to raise antibodies (or other affinity reagents) or indeed is capable of inducing an antibody response in a subject or experimental animal. “Immunogenic” is taken to mean that the protein is capable of eliciting an immune response such as a protective immune response in a subject or experimental animal. Thus, in the latter case, the protein may be capable of not only generating an antibody response but, in addition, non-antibody based immune responses. “Immunogenic” also embraces whether the protein may elicit an immune-like response in an in-vitro setting e.g. a T-cell proliferation assay. The generation of an appropriate immune response may require the presence of one or more adjuvants and/or appropriate presentation of an antigen.

[0267] The skilled person will appreciate that homologues or derivatives of OGTA066 will also find use as antigenic/immunogenic material and for other applications according to the invention. Thus, for instance proteins which include one or more additions, deletions, substitutions or the like are encompassed by the present invention. In addition, it may be possible to replace one amino acid with another of similar “type”. For instance, replacing one hydrophobic amino acid with another. One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of analysis are contemplated in the present invention.

[0268] In the case of homologues and derivatives, the degree of identity with a protein as described herein is less important than that the homologue or derivative should retain its antigenicity and/or immunogenicity. However, suitably, homologues or derivatives having at least 60% similarity (as discussed above) or identity with the proteins or polypeptides described herein are provided, for example, homologues or derivatives having at least 70% similarity or identity, such as at least 80% similarity or identity are provided. Particularly, homologues or derivatives having at least 90% or even 95% similarity or identity are provided.

[0269] In an alternative approach, the homologues or derivatives could be fusion proteins, incorporating moieties which render purification easier, for example by effectively tagging the desired protein or polypeptide. It may be necessary to remove the “tag” or it may be the case that the fusion protein itself retains sufficient antigenicity to be useful.

[0270] It is well known that it is possible to screen an antigenic protein or polypeptide to identify epitopic regions,

i.e. those regions which are responsible for the protein or polypeptide’s antigenicity or immunogenicity. Methods well known to the skilled person can be used to test fragments and/or homologues and/or derivatives for antigenicity. Thus, the fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a protein or polypeptide, homologue or derivative as described herein. The key issue, once again, is that the fragment retains the antigenic/immunogenic properties of the protein from which it is derived.

[0271] What is important for homologues, derivatives and fragments is that they possess at least a degree of the antigenicity/immunogenicity of the protein or polypeptide from which they are derived. Thus, in an additional aspect of the invention, there is provided antigenic/or immunogenic fragments of OGTA066 or of homologues or derivatives thereof.

[0272] OGTA066, or antigenic fragments thereof, can be provided alone, as a purified or isolated preparation. They may be provided as part of a mixture with one or more other proteins employed in the invention, or antigenic fragments thereof. In a further aspect, therefore, the invention provides an antigen composition comprising OGTA066 and/or one or more antigenic fragments thereof. Such a composition can be used for the detection and/or diagnosis of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0273] Vaccine compositions according to the invention may be either a prophylactic or therapeutic vaccine composition.

[0274] The vaccine compositions of the invention can include one or more adjuvants (immunostimulants). Examples well-known in the art include inorganic gels, such as aluminium hydroxide, and water-in-oil emulsions, such as incomplete Freund’s adjuvant. Other useful adjuvants will be well known to the skilled person.

[0275] Suitable adjuvants for use in vaccine compositions for the treatment of cancer include: 3De-O-acylated monophosphoryl lipid A (known as 3D-MPL or simply MPL see WO92/116556), a saponin, for example QS21 or QS7, and TLR4 agonists such as a CpG containing molecule, for example as disclosed in WO95/26204.

[0276] The adjuvants employed may be a combination of components, for example MPL and QS21 or MPL, QS21 and a CpG containing moiety.

[0277] Adjuvants may be formulated as oil-in-water emulsions or liposomal formulations.

[0278] In yet further aspects, the present invention provides:

[0279] (a) the use of OGTA066 an antigenic fragment thereof, or a composition comprising same in the preparation of an immunogenic composition, preferably a vaccine;

[0280] (b) the use of such an immunogenic composition in inducing an immune response in a subject; and

[0281] (c) a method for the treatment or prophylaxis of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, in a subject, which comprises the step of administering to the subject an effective amount of OGTA066 at least one antigenic fragment thereof or an immunogenic composition of the same, for example where the treatment is a vaccine and wherein the method of treatment is vaccination.

[0282] Thus in a specific embodiment, a preparation of OGTA066 or OGTA066 peptide fragments is used as a vac-

cine for the treatment of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Such preparations may include adjuvants or other vehicles.

[0283] In another embodiment, a preparation of oligonucleotides comprising 10 or more consecutive nucleotides complementary to a nucleotide sequence encoding OGTA066, or an OGTA066 peptide fragment is used as a vaccine for the treatment of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Such preparations may include adjuvants or other vehicles.

Inhibition of OGTA066 to Treat Colorectal Cancer, Kidney Cancer, Lung Cancer and Pancreatic Cancer

[0284] In one embodiment of the invention, colorectal cancer, kidney cancer, lung cancer or pancreatic cancer is treated or prevented by administration of a compound that antagonizes (inhibits) the level(s) and/or function(s) of OGTA066 which are elevated in the relevant serum or tissue of subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer as compared with similar serum or tissue of subjects free from colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0285] Compounds useful for this purpose include but are not limited to anti-OGTA066 antibodies (or other affinity reagents, and fragments and derivatives containing the binding region thereof), OGTA066 antisense or ribozyme nucleic acids, and nucleic acids encoding dysfunctional OGTA066 may be used to "knockout" endogenous OGTA066 function by homologous recombination (see, e.g., Capecchi, 1989, *Science* 244:1288-1292).

[0286] Other compounds that inhibit OGTA066 function can be identified by use of known in vitro assays, e.g., assays for the ability of a test compound to inhibit binding of OGTA066 to another protein or a binding partner, or to inhibit a known OGTA066 function.

[0287] Such inhibition may, for example be assayed in vitro or in cell culture, but genetic assays may also be employed. The Preferred Technologies described in Examples 1 and 2 can also be used to detect levels of OGTA066 before and after the administration of the compound. Suitable in vitro or in vivo assays are utilized to determine the effect of a specific compound and whether its administration is indicated for treatment of the affected tissue.

[0288] In a specific embodiment, a compound that inhibits OGTA066 function (activity) is administered therapeutically or prophylactically to a subject in whom an increased serum or tissue level or functional activity of OGTA066 (e.g., greater than the normal level or desired level) is detected as compared with serum or tissue of subjects with colorectal cancer, kidney cancer, lung cancer and/or pancreatic cancer who do not receive treatment according to the invention or to bring the level or activity to that found in subjects free from said cancers. Methods standard in the art can be employed to measure the increase in OGTA066 level or function, as outlined above. Suitable OGTA066 inhibitor compositions may for example include small molecules, i.e. molecules of 1000 daltons or less. Such small molecules can be identified by the screening methods described herein.

Assays for Therapeutic or Prophylactic Compounds

[0289] The present invention also provides assays for use in drug discovery in order to identify or verify the efficacy of

compounds for treatment or prevention of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0290] Thus there is provided a method of screening for compounds that modulate the activity of OGTA066, the method comprising: (a) contacting OGTA066 or a biologically active portion thereof with a candidate compound; and (b) determining whether activity of OGTA066 is thereby modulated. Such a process may comprise (a) contacting OGTA066 or a biologically active portion thereof with a candidate compound in a sample; and (b) comparing the activity of OGTA066 or a biologically active portion thereof in said sample after contact with said candidate compound with the activity of OGTA066 or a biologically active portion thereof in said sample before contact with said candidate compound, or with a reference level of activity.

[0291] The method of screening may be a method of screening for compounds that inhibit activity of OGTA066.

[0292] OGTA066 or a biologically active portion thereof may, for example be expressed on or by a cell. OGTA066 or a biologically active portion thereof may, for example, be isolated from cells which express it. OGTA066 or a biologically active portion thereof may, for example, be immobilised onto a solid phase.

[0293] There is also provided a method of screening for compounds that modulate the expression of OGTA066 or nucleic acid encoding OGTA066, the method comprising: (a) contacting cells expressing OGTA066 or nucleic acid encoding OGTA066 with a candidate compound; and (b) determining whether expression of OGTA066 or nucleic acid encoding OGTA066 is thereby modulated. Such a process may comprise (a) contacting cells expressing OGTA066 or nucleic acid encoding OGTA066 with a candidate compound in a sample; and (b) comparing the expression of OGTA066 or nucleic acid encoding OGTA066 by cells in said sample after contact with said candidate compound with the expression of OGTA066 or nucleic acid encoding OGTA066 of cells in said sample before contact with said candidate compound, or with a reference level of expression.

[0294] The method may be a method of screening for compounds that inhibit expression of OGTA066 or nucleic acid encoding OGTA066.

[0295] Other aspects of the invention include: a compound obtainable by an aforementioned screening method, a compound which modulates the activity or expression of OGTA066 or nucleic acid encoding OGTA066, for example a compound which inhibits the activity or expression of OGTA066 or nucleic acid encoding OGTA066.

[0296] Such a compound is provided for use in treating or preventing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. There is also provided a method for treating or preventing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer which comprises administering to a subject in need thereof a therapeutically effective amount of such a compound.

[0297] Test compounds can be assayed for their ability to restore OGTA066 levels in a subject having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer towards levels found in subjects free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer or to produce similar changes in experimental animal models of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Compounds able to restore OGTA066 levels in a subject having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer towards levels found in subjects free from colorectal

cancer, kidney cancer, lung cancer and pancreatic cancer or to produce similar changes in experimental animal models of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer can be used as lead compounds for further drug discovery, or used therapeutically. OGTA066 expression can be assayed by the Preferred Technologies, immunoassays, gel electrophoresis followed by visualization, detection of OGTA066 activity, or any other method taught herein or known to those skilled in the art. Such assays can be used to screen candidate drugs, in clinical monitoring or in drug development, where abundance of OGTA066 can serve as a surrogate marker for clinical disease.

[0298] In various specific embodiments, *in vitro* assays can be carried out with cells representative of cell types involved in a subject's disorder, to determine if a compound has a desired effect upon such cell types.

[0299] Compounds for use in therapy can be tested in suitable animal model systems prior to testing in humans, including but not limited to rats, mice, chicken, cows, monkeys, rabbits, etc. For *in vivo* testing, prior to administration to humans, any animal model system known in the art may be used. Examples of animal models of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer include, but are not limited to xenografts of human colorectal cancer cell lines such as MDA-MB-345 in oestrogen-deprived SCID mice, Eccles et al. 1994 *Cell Biophysics* 24/25, 279; xenografts of renal cell cancer cell lines such as LABAZ1 in immune compromised mice, Zisman et al, *Cancer Research* 63, 4952-4959, Aug. 15, 2003 or xenografts of non small cell lung cancer cell lines such as A549 and H460 and xenografts of small cell lung cancer cell lines such as NCI-H345 or xenografts of pancreatic cancer cell lines such as MIA PaCa-2 in nude mice, Marincola et al., *J Surg Res* 1989 December; 47(6):520-9. These can be utilized to test compounds that modulate OGTA066 levels, since the pathology exhibited in these models is similar to that of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. It is also apparent to the skilled artisan that based upon the present disclosure, transgenic animals can be produced with "knock-out" mutations of the gene or genes encoding OGTA066. A "knock-out" mutation of a gene is a mutation that causes the mutated gene to not be expressed, or expressed in an aberrant form or at a low level, such that the activity associated with the gene product is nearly or entirely absent. Preferably, the transgenic animal is a mammal; more preferably, the transgenic animal is a mouse.

[0300] In one embodiment, test compounds that modulate the expression of OGTA066 are identified in non-human animals (e.g. mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, expressing OGTA066. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of the test compound on expression of OGTA066 is determined. A test compound that alters the expression of OGTA066 can be identified by comparing the level of OGTA066 (or mRNA encoding the same) in an animal or group of animals treated with a test compound with the level of OGTA066 or mRNA in an animal or group of animals treated with a control compound. Techniques known to those of skill in the art can be used to determine the mRNA and protein levels, for example, *in situ* hybridization. The animals may or may not be sacrificed to assay the effects of a test compound.

[0301] In another embodiment, test compounds that modulate the activity of OGTA066 or a biologically active portion thereof are identified in non-human animals (e.g. mice, rats, monkeys, rabbits, and guinea pigs), preferably non-human animal models for colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, expressing OGTA066. In accordance with this embodiment, a test compound or a control compound is administered to the animals, and the effect of a test compound on the activity of OGTA066 is determined. A test compound that alters the activity of OGTA066 can be identified by assaying animals treated with a control compound and animals treated with the test compound. The activity of OGTA066 can be assessed by detecting induction of a cellular second messenger of OGTA066 (e.g. intracellular Ca^{2+} , diacylglycerol, IP3, etc.), detecting catalytic or enzymatic activity of OGTA066 or binding partner thereof, detecting the induction of a reporter gene (e.g. a regulatory element that is responsive to OGTA066 operably linked to a nucleic acid encoding a detectable marker, such as luciferase or green fluorescent protein), or detecting a cellular response (e.g. cellular differentiation or cell proliferation). Techniques known to those of skill in the art can be utilized to detect changes in the activity of OGTA066 (see, e.g. U.S. Pat. No. 5,401,639, which is incorporated herein by reference).

[0302] In yet another embodiment, test compounds that modulate the level or expression of OGTA066 are identified in human subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, preferably those having severe colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. In accordance with this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on OGTA066 expression is determined by analyzing the expression of OGTA066 or the mRNA encoding the same in a biological sample (e.g. serum, plasma, or urine). A test compound that alters the expression of OGTA066 can be identified by comparing the level of OGTA066 or mRNA encoding the same in a subject or group of subjects treated with a control compound to that in a subject or group of subjects treated with a test compound. Alternatively, alterations in the expression of OGTA066 can be identified by comparing the level of OGTA066 or mRNA encoding the same in a subject or group of subjects before and after the administration of a test compound. Techniques known to those of skill in the art can be used to obtain the biological sample and analyze the mRNA or protein expression. For example, the Preferred Technologies described in Examples 1 and 2 described herein can be used to assess changes in the level of OGTA066.

[0303] In another embodiment, test compounds that modulate the activity of OGTA066 are identified in human subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer (preferably those with severe colorectal cancer, kidney cancer, lung cancer or pancreatic cancer). In this embodiment, a test compound or a control compound is administered to the human subject, and the effect of a test compound on the activity of OGTA066 is determined. A test compound that alters the activity of OGTA066 can be identified by comparing biological samples from subjects treated with a control compound to samples from subjects treated with the test compound. Alternatively, alterations in the activity of OGTA066 can be identified by comparing the activity of OGTA066 in a subject or group of subjects before and after the administration of a test compound. The activity of OGTA066 can be assessed by detecting in a biological sample

(e.g. serum, plasma, or urine) induction of a cellular signal transduction pathway of OGTA066 (e.g. intracellular Ca^{2+} , diacylglycerol, IP3, etc.), catalytic or enzymatic activity of OGTA066 or a binding partner thereof, or a cellular response, for example, cellular differentiation, or cell proliferation. Techniques known to those of skill in the art can be used to detect changes in the induction of a second messenger of OGTA066 or changes in a cellular response. For example, RT-PCR can be used to detect changes in the induction of a cellular second messenger.

[0304] In a preferred embodiment, a test compound that changes the level or expression of, OGTA066 towards levels detected in control subjects (e.g. humans free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer) is selected for further testing or therapeutic use. In another preferred embodiment, a test compound that changes the activity of OGTA066 towards the activity found in control subjects (e.g., humans free from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer) is selected for further testing or therapeutic use.

[0305] In another embodiment, test compounds that reduce the severity of one or more symptoms associated with colorectal cancer, kidney cancer, lung cancer or pancreatic cancer are identified in human subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, preferably subjects with severe colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. In accordance with this embodiment, a test compound or a control compound is administered to the subjects, and the effect of a test compound on one or more symptoms of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer is determined. A test compound that reduces one or more symptoms can be identified by comparing the subjects treated with a control compound to the subjects treated with the test compound. Techniques known to physicians familiar with colorectal cancer, kidney cancer, lung cancer or pancreatic cancer can be used to determine whether a test compound reduces one or more symptoms associated with colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. For example, a test compound that reduces tumour burden in a subject having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer will be beneficial for subjects having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

[0306] In another embodiment, a test compound that reduces the severity of one or more symptoms associated with colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, in a human having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, is selected for further testing or therapeutic use.

Therapeutic and Prophylactic Compositions and their Use

[0307] The invention provides methods of treatment (and prophylaxis) comprising administering to a subject an effective amount of a compound employed in the invention. In a particular aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is for example an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is for example a mammal, such as a human. In a specific embodiment, a non-human mammal is the subject.

[0308] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid are described above; additional appropriate formulations and routes of administration are described below.

[0309] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction can be enteral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0310] In one aspect of the invention a nucleic acid employed in the invention may be delivered to the dermis, for example employing particle mediated epidermal delivery.

[0311] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved, for example, and not by way of limitation, by local infusion during surgery, topical application, e.g., by injection, by means of a catheter, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

[0312] In one embodiment, administration can be by direct injection into colorectal, kidney, lung or pancreatic tissue or at the site (or former site) of a malignant tumour or neoplastic or pre-neoplastic tissue.

[0313] In another embodiment, the compound can be delivered in a vesicle, in particular a liposome (see Langer, 1990, *Science* 249:1527-1533; Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*).

[0314] In yet another embodiment, the compound can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:201; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, *N. Engl. J. Med.* 321:574).

[0315] In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla. (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., 1983, *Macromol. Sci. Rev. Macromol. Chem.* 23:61; see also Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 71:105). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g. the colon, kidney, lung or pancreas thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138 (1984)).

[0316] Other controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533).

[0317] In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[0318] The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier.

[0319] In a specific embodiment, the term "pharmaceutically acceptable" means suitable for approval by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0320] The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the compound, for example in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

[0321] In a one embodiment for example where one or more antibodies are employed, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solu-

bilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent.

[0322] Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0323] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts, where appropriate, include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0324] The amount of the compound of the invention which will be effective in the treatment of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each subject's circumstances. However, suitable dosage ranges for intravenous administration are generally about 20-500 micrograms of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 pg/kg body weight to 1 mg/kg body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0325] Suppositories generally contain active ingredient in the range of 0.5% to 10% by weight; oral formulations preferably contain 10% to 95% active ingredient.

[0326] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

[0327] Thus in one aspect the kit comprises antibodies employed in the invention, for example the antibodies may be lyophilized for reconstitution before administration or use. Where the kit is for use in therapy/treatment such as cancer the antibody or antibodies may be reconstituted with an isotonic aqueous solution, which may optionally be provided with the kit. In one aspect the kit may comprise a polypeptide such as an immunogenic polypeptide employed in the invention, which may for example be lyophilized. The latter kit may further comprise an adjuvant for reconstituting the immunogenic polypeptide.

[0328] The invention also extends to a composition as described herein for example a pharmaceutical composition and/or vaccine compositions for use in inducing an immune response in a subject.

[0329] The invention also relates to an antibody, kit, composition, method, use or vaccine according to any one of as described herein wherein OGTA066 is 1, 2, 3, 4 or 5 sequences defined by SEQ ID Nos 1-5.

[0330] For clarity of disclosure, and not by way of limitation, the invention will be described with respect to the analysis of colorectal, kidney, lung and pancreatic tissue. However, as one skilled in the art will appreciate, the assays and techniques described below can be applied to other types of patient samples, including body fluids (e.g. blood, urine or saliva), a tissue sample from a patient at risk of having colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, (e.g. a biopsy such as a colorectal, kidney, lung or pancreatic biopsy) or homogenate thereof.

Determining Abundance of OGTA066 by Imaging Technology

[0331] An advantage of determining abundance of OGTA066 by imaging technology may be that such a method is non-invasive (save that reagents may need to be administered) and there is no need to extract a sample from the subject.

[0332] Suitable imaging technologies include positron emission tomography (PET) and single photon emission computed tomography (SPECT). Visualisation of OGTA066 using such techniques requires incorporation or binding of a suitable label e.g. a radiotracer such as ^{18}F , ^{11}C or ^{123}I (see e.g. NeuroRx—The Journal of the American Society for Experimental NeuroTherapeutics (2005) 2(2), 348-360 and idem pages 361-371 for further details of the techniques). Radiotracers or other labels may be incorporated into OGTA066 by administration to the subject (e.g. by injection) of a suitably labelled specific ligand. Alternatively they may be incorporated into a binding affinity reagent (e.g. antibody) specific for OGTA066 which may be administered to the subject (e.g. by injection). For discussion of use of Affibodies for imaging see e.g. Orlova A, Magnusson M, Eriksson T L, Nilsson M, Larsson B, Hoiden-Guthenberg I, Widstrom C, Carlsson J, Tolmachev V, Stahl S, Nilsson FY, Tumor imaging using a picomolar affinity HER2 binding Affibody molecule, Cancer Res. 2006 Apr. 15; 66(8):4339-48).

Diagnosis and Treatment of Colorectal Cancer, Kidney Cancer, Lung Cancer or Pancreatic Cancer Using Immunohistochemistry

[0333] Immunohistochemistry is an excellent detection technique and may therefore be very useful in the diagnosis and treatment of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer. Immunohistochemistry may be used to detect, diagnose, or monitor colorectal cancer, kidney cancer, lung cancer or pancreatic cancer through the localization of OGTA066 antigens in tissue sections by the use of labeled antibodies (or other affinity reagents), derivatives and analogs thereof, which specifically bind to OGTA066, as specific reagents through antigen-antibody interactions that are visualized by a marker such as fluorescent dye, enzyme, radioactive element or colloidal gold.

[0334] The advancement of monoclonal antibody technology has been of great significance in assuring the place of immunohistochemistry in the modern accurate microscopic diagnosis of human neoplasms. The identification of disseminated neoplastically transformed cells by immunohistochemistry allows for a clearer picture of cancer invasion and

metastasis, as well as the evolution of the tumour cell associated immunophenotype towards increased malignancy. Future antineoplastic therapeutical approaches may include a variety of individualized immunotherapies, specific for the particular immunophenotypical pattern associated with each individual patient's neoplastic disease. For further discussion see e.g. Bodey B, The significance of immunohistochemistry in the diagnosis and therapy of neoplasms, Expert Opin Biol Ther. 2002 April; 2(4):371-93.

[0335] Preferred features of each aspect of the invention are as for each of the other aspects mutatis mutandis. The prior art documents mentioned herein are incorporated to the fullest extent permitted by law.

Example 1

Identification of Membrane Proteins Expressed in Colorectal Cancer and Pancreatic Cancer Blood and Tissue Samples Using 1D Gel Electrophoresis

[0336] Using the following Reference Protocol, membrane proteins extracted from colorectal cancer and pancreatic cancer tissue samples were separated by 1D gel and analysed.

1.1 Materials and Methods

1.1.1—Plasma Membrane Fractionation

[0337] The cells recovered from a colorectal cancer or pancreatic cancer were lysed and submitted to centrifugation at 1000 G. The supernatant was taken, and it was subsequently centrifuged at 3000 G. Once again, the supernatant was taken, and it was then centrifuged at 100 000 G.

[0338] The resulting pellet was recovered and put on 15-60% sucrose gradient.

[0339] A Western blot was used to identify sub cellular markers, and the Plasma Membrane fractions were pooled.

[0340] The pooled solution was either run directly on 1D gels (see section 1.1.4 below), or further fractionated into heparin binding and nucleotide binding fractions as described below.

1.1.2—Plasma Membrane Heparin-binding Fraction

[0341] The pooled solution from 1.1.1 above was applied to a Heparin column, eluted from column and run on 1D gels (see section 1.1.4 below).

1.1.3—Plasma Nucleotide-Binding Fraction

[0342] The pooled solution from 1.1.1 above was applied to a Cibacrom Blue 3GA column, eluted from column and run on 1D gels (see section 1.1.4 below).

1.1.4—1D Gel Technology

[0343] Protein or membrane pellets were solubilised in 1D sample buffer (1-2 $\mu\text{g}/\mu\text{l}$). The sample buffer and protein mixture was then heated to 95° C. for 3 min.

[0344] A 9-16% acrylamide gradient gel was cast with a stacking gel and a stacking comb according to the procedure described in Ausubel F. M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. II, Green Publishing Associates, Inc., and John Wiley & Sons, Inc., New York, section 10.2, incorporated herein by reference in its entirety.

[0345] 30-50 micrograms of the protein mixtures obtained from detergent and the molecular weight standards (66, 45,

31, 21, 14 kDa) were added to the stacking gel wells using a 10 microlitre pipette tip and the samples run at 40 mA for 5 hours.

[0346] The plates were then prised open, the gel placed in a tray of fixer (10% acetic acid, 40% ethanol, 50% water) and shaken overnight. Following this, the gel was primed by 30 minutes shaking in a primer solution (7.5% acetic acid (75 ml), 0.05% SDS (5 ml of 10%)). The gel was then incubated with a fluorescent dye (7.5% acetic acid, 0.06% OGS in-house dye (6000) with shaking for 3 hrs. Sypro Red (Molecular Probes, Inc., Eugene, Oreg.) is a suitable dye for this purpose. A preferred fluorescent dye is disclosed in U.S. application Ser. No. 09/412,168, filed on Oct. 5, 1999, which is incorporated herein by reference in its entirety.

[0347] A computer-readable output was produced by imaging the fluorescently stained gels with an Apollo 3 scanner (Oxford Glycosciences, Oxford, UK). This scanner is developed from the scanner described in WO 96/36882 and in the Ph.D. thesis of David A. Basiji, entitled "Development of a High-throughput Fluorescence Scanner Employing Internal Reflection Optics and Phase-sensitive Detection (Total Internal Reflection, Electrophoresis)", University of Washington (1997), Volume 58/12-B of Dissertation Abstracts International, page 6686, the contents of each of which are incorporated herein by reference. The latest embodiment of this instrument includes the following improvements: The gel is transported through the scanner on a precision lead-screw drive system. This is preferable to laying the glass plate on the belt-driven system that is defined in the Basiji thesis as it provides a reproducible means of accurately transporting the gel past the imaging optics.

[0348] The gel is secured into the scanner against three alignment stops that rigidly hold the glass plate in a known position. By doing this in conjunction with the above precision transport system and the fact that the gel is bound to the glass plate, the absolute position of the gel can be predicted and recorded. This ensures that accurate co-ordinates of each feature on the gel can be communicated to the cutting robot for excision. This cutting robot has an identical mounting arrangement for the glass plate to preserve the positional accuracy.

[0349] The carrier that holds the gel in place has integral fluorescent markers (Designated M1, M2, M3) that are used to correct the image geometry and are a quality control feature to confirm that the scanning has been performed correctly.

[0350] The optical components of the system have been inverted. The laser, mirror, waveguide and other optical components are now above the glass plate being scanned. The embodiment of the Basiji thesis has these underneath. The glass plate is therefore mounted onto the scanner gel side down, so that the optical path remains through the glass plate. By doing this, any particles of gel that may break away from the glass plate will fall onto the base of the instrument rather than into the optics.

[0351] In scanning the gels, they were removed from the stain, rinsed with water and allowed to air dry briefly and imaged on the Apollo 3. After imaging, the gels were sealed in polyethylene bags containing a small volume of staining solution, and then stored at 4° C.

[0352] Apparent molecular weights were calculated by interpolation from a set of known molecular weight markers run alongside the samples.

1.1.5—Recovery and Analysis of Selected Proteins

[0353] Proteins were robotically excised from the gels by the process described in U.S. Pat. No. 6,064,754, Sections 5.4

and 5.6, 5.7, 5.8 (incorporated herein by reference), as is applicable to 1D-electrophoresis, with modification to the robotic cutter as follows: the cutter begins at the top of the lane, and cuts a gel disc 1.7 mm in diameter from the left edge of the lane. The cutter then moves 2 mm to the right, and 0.7 mm down and cuts a further disc. This is then repeated. The cutter then moves back to a position directly underneath the first gel cut, but offset by 2.2 mm downwards, and the pattern of three diagonal cuts are repeated. This is continued for the whole length of the gel.

[0354] NOTE: If the lane is observed to broaden significantly then a correction can be made also sideways i.e. instead of returning to a position directly underneath a previous gel cut, the cut can be offset slightly to the left (on the left of the lane) and/or the right (on the right of the lane). The proteins contained within the gel fragments were processed to generate tryptic peptides; partial amino acid sequences of these peptides were determined by mass spectroscopy as described in WO98/53323 and application Ser. No. 09/094,996, filed Jun. 15, 1998.

[0355] Proteins were processed to generate tryptic digest peptides. Tryptic peptides were analyzed by mass spectrometry using a PerSeptive Biosystems Voyager-DETM STR Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometer, and selected tryptic peptides were analyzed by tandem mass spectrometry (MS/MS) using a Micromass Quadrupole Time-of-Flight (Q-TOF) mass spectrometer (Micromass, Altrincham, U.K.) equipped with a Nanoflow™ electrospray Z-spray source. For partial amino acid sequencing and identification of OGTA066 uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program (Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989), version v.C.1. Criteria for database identification included: the cleavage specificity of trypsin; the detection of a suite of a, b and y ions in peptides returned from the database, and a mass increment for all Cys residues to account for carbamidomethylation. The database searched was a database constructed of protein entries in the non-redundant database held by the National Centre for Biotechnology Information (NCBI) which is accessible at www.ncbi.nlm.nih.gov/. Following identification of proteins through spectral-spectral correlation using the SEQUEST program, masses detected in MALDI-TOF mass spectra were assigned to tryptic digest peptides within the proteins identified. In cases where no amino acid sequences could be identified through searching with uninterpreted MS/MS spectra of tryptic digest peptides using the SEQUEST program, tandem mass spectra of the peptides were interpreted manually, using methods known in the art. (In the case of interpretation of low-energy fragmentation mass spectra of peptide ions see Gaskell et al., 1992, Rapid Commun. Mass Spectrom. 6:658-662).

1.1.6—Discrimination of Colorectal Cancer and Pancreatic Cancer, Associated Proteins

[0356] The process to identify OGTA066 uses the peptide sequences obtained experimentally by mass spectrometry described above of naturally occurring human proteins to identify and organise coding exons in the published human genome sequence.

[0357] Recent dramatic advances in defining the chemical sequence of the human genome have led to the near completion of this immense task (Venter, J. C. et al. (2001). The sequence of the human genome. *Science* 16: 1304-51; International Human Genome Sequencing Consortium. (2001). Initial sequencing and analysis of the human genome *Nature* 409: 860-921). There is little doubt that this sequence information will have a substantial impact on our understanding of many biological processes, including molecular evolution, comparative genomics, pathogenic mechanisms and molecular medicine. For the full medical value inherent in the sequence of the human genome to be realised, the genome needs to be 'organised' and annotated. By this, is meant at least the following three things: (i) The assembly of the sequences of the individual portions of the genome into a coherent, continuous sequence for each chromosome. (ii) The unambiguous identification of those regions of each chromosome that contain genes. (iii) Determination of the fine structure of the genes and the properties of its mRNA and protein products. While the definition of a 'gene' is an increasingly complex issue (H Pearson: What is a gene? *Nature* (2006) 24: 399-401), what is of immediate interest for drug discovery and development is a catalogue of those genes that encode functional, expressed proteins. A subset of these genes will be involved in the molecular basis of most if not all pathologies. Therefore an important and immediate goal for the pharmaceutical industry is to identify all such genes in the human genome and describe their fine structure.

Processing and Integration of Peptide Masses, Peptide Signatures, ESTs and Public Domain Genomic Sequence Data to Form OGAP® Database

[0358] Discrete genetic units (exons, transcripts and genes) were identified using the following sequential steps:

A 'virtual transcriptome' is generated, containing the tryptic peptides which map to the human genome by combining the gene identifications available from Ensembl and various gene prediction programs. This also incorporates SNP data (from dbSNP) and all alternate splicing of gene identifications. Known contaminants were also added to the virtual transcriptome.

All tandem spectra in the OGeS Mass Spectrometry Database are interpreted in order to produce a peptide that can be mapped to one in the virtual transcriptome. A set of automated spectral interpretation algorithms were used to produce the peptide identifications.

The set of all mass-matched peptides in the OGeS Mass Spectrometry Database is generated by searching all peptides from transcripts hit by the tandem peptides using a tolerance based on the mass accuracy of the mass spectrometer, typically 20 ppm.

All tandem and mass-matched peptides are combined in the form of "protein clusters". This is done using a recursive process which groups sequences into clusters based on common peptide hits. Biological sequences are considered to belong to the same cluster if they share one or more tandem or mass-matched peptide.

After initial filtering to screen out incorrectly identified peptides, the resulting clusters are then mapped on the human genome.

The protein clusters are then aggregated into regions that define preliminary gene boundaries using their proximity and the co-observation of peptides within protein clusters. Proximity is defined as the peptide being within 80,000 nucleotides on the same strand of the same chromosome. Various elimination rules, based on cluster observation scoring and multiple mapping to the genome are used to refine the output. The resulting 'confirmed genes' are those which best account for the peptides and masses observed by mass spectrometry in each cluster. Nominal co-ordinates for the gene are also an output of this stage.

The best set of transcripts for each confirmed gene are created from the protein clusters, peptides, ESTs, candidate exons and molecular weight of the original protein spot.

Each identified transcript was linked to the sample providing the observed peptides.

Use of an application for viewing and mining the data. The result of steps 1-8 was a database containing genes, each of which consisted of a number of exons and one or more transcripts. An application was written to display and search this integrated genome/proteome data. Any features (OMIM disease locus, InterPro etc.) that had been mapped to the same Golden Path co-ordinate system by Ensembl could be cross-referenced to these genes by coincidence of location and fine structure.

Results

[0359] The process was used to generate approximately 1 million peptide sequences to identify protein-coding genes and their exons resulted in the identification of protein sequences for 18083 genes across 67 different tissues and 57 diseases including 2,025 genes in acute T-cell leukaemia, 501 genes in B-cell non-Hodgkin's lymphoma, 4,713 genes in breast cancer, 1,371 genes in cervical cancer, 2,424 genes in chronic lymphocytic leukaemia, 949 genes in colorectal cancer, 524 genes in gastric cancer, 1,544 genes in glioblastoma, 1,782 genes in hepatocellular carcinoma, 978 genes in lung cancer, 373 genes in lymphoid leukaemia (unspecified), 1,764 genes in melanoma, 1,391 genes in neuroblastoma, 1,324 genes in osteosarcoma, 1,033 genes in ovarian cancer, 2,961 genes in pancreatic cancer, 3,307 genes in prostate cancer, 1005 genes in renal cell cancer and 1,783 genes in retinoblastoma, illustrated here by OGTA066 isolated and identified from colorectal cancer, kidney cancer, lung cancer and pancreatic cancer samples. Following comparison of the experimentally determined sequences with sequences in the OGAP® database, OGTA066 showed a high degree of specificity to colorectal cancer, kidney cancer, lung cancer and pancreatic cancer.

1.2 Results

[0360] These experiments identified OGTA066 as further described herein.

[0361] The full-length OGTA066 was detected in the plasma membrane of colorectal cancer and pancreatic cancer samples and was not detected in the cytosol.

[0362] FIG. 2 shows the Protein Index for OGTA066. For each gene, the protein index uses the mass spectrometry data to assign a score to each disease, relative to the global database. The Protein Index can then be used to identify cancer specific genes with a high score in cancer indications and low/negligible scores in normal and other diseases. The index contains ~1 million peptides sequenced via mass spectrometry.

etry from 56 diseases. For each gene, this yields a score for each disease and subcellular location. The results are summarized below:

Protein Index Report for OGTA066

[0363] Indications positive:

Colorectal cancer

Kidney cancer

Lung cancer

Pancreatic cancer

Disease controls
Acute monocytic leukaemia
Acute T-cell leukaemia
Alzheimer's Disease
Arthritis
Asthma
Atherosclerosis
B-cell non-Hodgkin's lymphoma
Bladder carcinoma
Breast cancer
Breast diseases, benign
Burkitt's lymphoma
Bursitis
Cancer, unspecified
Cervical cancer
Chronic lymphocytic leukaemia
Chronic obstructive pulmonary disease
<u>Colorectal cancer</u>
Dementia, vascular
Depression
Diabetes and Obesity
Diverticulitis
Dyslipidaemia
Emphysema
Focal apocrine metaplasia
Gastric cancer
Gaucher disease
Glioblastoma
Hepatoblastoma
Hepatocellular carcinoma
Hypertension
Lactational foci
<u>Kidney cancer</u>
Leukaemia, unspecified
Liver cirrhosis
<u>Lung cancer</u>
Lymphoma, histiocytic
Melanoma
Metabolic syndrome X
Migraine, acute
Multiple sclerosis
Neuroblastoma
<u>Normal</u>
Obesity
Osteoarthritis
Osteosarcoma
Ovarian cancer
Pancreatic cancer
<u>Prostate cancer</u>
Prostatic diseases, benign
Prostatitis
Retinoblastoma

-continued

Disease controls
Schizophrenia
Skin ulcer
Smoker
Teratocarcinoma
Subcellular location
Birbeck Granules
Cell surface digest
Chromatin Fraction
Crude Cell Membrane
Cytosol
Golgi/Mitochondrial Membrane
Membrane Glycoprotein Binding Fraction
Mitochondria
Nucleus
Peroxisomes
<u>Plasma Membrane</u>
Secreted
Soluble Fraction
Supernatant
Whole Cell

[0364] FIG. 2 shows the Protein Index for OGTA066 is very high in colorectal cancer, low in kidney cancer, high in lung cancer, high in pancreatic cancer and very low in normal plasma membrane. OGTA066 was also detected as low in normal membrane samples. OGTA066 was not detected in any other diseases. This indicates that OGTA066 is potentially a good marker for colorectal cancer, kidney cancer, lung cancer and pancreatic cancer.

Example 2

Identification of Membrane Proteins Expressed in Colorectal Cancer, Kidney Cancer or Lung Cancer Blood and Tissue Samples Using Isotope tagging for absolute and relative quantitation (iTRAQ)

[0365] Using the following Reference Protocols, membrane proteins extracted from colorectal cancer, kidney cancer and lung cancer tissue and normal adjacent colorectal, kidney and lung tissue samples were digested, labelled with Isotope Tagging for Absolute & Relative Quantitation reagents (iTRAQ; Applied Biosystems, Foster City, Calif., USA) and resulting peptides sequenced by tandem mass spectrometry.

2.1 Materials and Methods

2.1.1 Plasma Membrane Fractionation

[0366] The cells recovered from a colorectal cancer, kidney cancer or lung cancer or normal adjacent colorectal, kidney or lung tissue were lysed and submitted to centrifugation at 1000 G. The supernatant was taken, and it was subsequently centrifuged at 3000 G. Once again, the supernatant was taken, and it was then centrifuged at 100 000 G.

[0367] The resulting pellet was recovered and put on 15-60% sucrose gradient.

[0368] A Western blot was used to identify sub cellular markers, and the Plasma Membrane fractions were pooled.

[0369] The pooled solution was then analysed directly by iTRAQ (see section 2.1.2 below).

2.1.2—iTRAQ Methodology

[0370] Membrane protein pellets from colorectal cancer, kidney cancer or lung cancer and normal adjacent colorectal, kidney or lung tissue were solubilised in sample buffer (2-4 µg/µl in 0.5% SDS) by the addition of buffer and then heating to 95° C. for 3 min.

[0371] To a volume of each protein solution equating to 50 µg, 150 µl of 0.5M triethylammonium bicarbonate (TEAB) solution was added. To each sample, 3 µl of 50 mM tris-(2-carboxyethyl)phosphine was added and the mixture was incubated at 60° C. for 1 hour. 1 µl of cysteine blocking reagent, 200 mM methyl methanethiosulphonate (MMTS) in isopropanol, was then added. After incubation at room temperature for 10 minutes, 15 µl of 1 µg/µl trypsin was added to each sample followed by incubation at 37° C. overnight.

[0372] The digested samples were dried under a vacuum and re-constituted with 30 µl of 0.5M TEAB solution. 70 µl ethanol was added to each of the four iTRAQ reagents (114/115/116/117) and one reagent added to each of the four samples analysed (two colorectal cancer, kidney cancer or lung cancer samples and two corresponding normal adjacent tissue samples) and left at room temperature for 1 hour. The specific reagent added to each sample was recorded. The four labeled samples were combined & vortexed.

[0373] The combined sample was reduced to dryness under a vacuum and de-salted by loading onto a C18 spin column, washing with aqueous solvent and then eluting with 70% acetonitrile. The sample fraction was again reduced to dryness and then re-dissolved in 40 µl of solvent A (97.9 water, 2% acetonitrile, 0.1% formic acid) prior to ion exchange fractionation.

2.1.3—Fractionation and Analysis of Labeled Peptides

[0374] The sample was fractionated by strong cation exchange chromatography using an Agilent 1200 chromatograph (Agilent, Santa Clara, Calif., USA). Samples were eluted off an Agilent Zorbax Bio-SCXII column (3.5 µm; 50x0.8 mm) using a 200 min gradient of 0-100 mM sodium acetate over 20 minutes and then to 1M over 10 minutes. 1 minute fractions were collected over the 30 minute run.

[0375] Each fraction was analysed by liquid chromatography/mass spectrometry using an Agilent 1200 chromatograph fitted with a Zorbax 300SB-C18 (150 mmx75 µm) and an Agilent 6510 quadrupole-time-of-flight instrument (Agilent, Santa Clara, Calif., USA). Peptides were eluted with a 300 nl/min gradient increasing from 15% to 45% acetonitrile in 60 minutes. Data was acquired in auto MS/MS mode such that up to 3 precursor ions above the intensity threshold were selected and product ion spectra accumulated to facilitate the sequencing of the labeled peptides. Raw was processed to create peak lists using Spectrum Mill software (Agilent, Santa Clara, Calif., USA).

2.1.4—Amino Acid Sequence Analysis of Labeled Peptides

[0376] For partial amino acid sequencing and identification of OGTA066, uninterpreted tandem mass spectra of tryptic peptides were searched using the SEQUEST search program

(Eng et al., 1994, J. Am. Soc. Mass Spectrom. 5:976-989). Criteria for database identification included: the cleavage specificity of trypsin; the detection of a suite of a, b and y ions in peptides returned from the database, and a mass increment for all cysteine residues to account for modification with methyl methanethiosulphonate and the addition of iTRAQ labels to free amines (N-terminus & lysine). The data was searched through IPI Human v3.23 (www.ebi.ac.uk/IPI/IPI-human.html).

[0377] 2.1.5—Discrimination of Colorectal Cancer, Kidney Cancer or Lung Cancer Associated Proteins

[0378] The process described in Example 1 section 1.1.6 was employed to discriminate the colorectal cancer, kidney cancer and lung cancer associated proteins in the experimental samples.

2.2 RESULTS

[0379] These experiments identified OGTA066, as further described herein. The full-length OGTA066 was detected in the plasma membrane of colorectal cancer, kidney cancer and lung cancer samples. The iTRAQ analysis showed that levels of OGTA066 in the cancer samples were higher than in the matched normal adjacent tissue samples.

[0380] FIG. 2 shows the Protein Index for OGTA066. See Example 1 section 1.2 for a description of the Protein Index for OGTA066.

Example 3

Evaluation of Colorectal Cancer Marker Proteins in Sandwich ELISA

[0381] Using the following Reference Protocol, colorectal cancer marker proteins, including OGTA066 were evaluated in a sandwich ELISA.

3.1 Materials and Methods

[0382] Antibodies for the sandwich ELISAs were developed at Biosite. Biotinylated antibody (primary antibody) was diluted into assay buffer (10 mM Tris, 150 mM NaCl, 1% BSA) to 2 µg/ml and added to 384 well neutravidin coated plate (Pierce Chemical Company, Rockford Ill.) and allowed to incubate at room temperature for 1 hour. Wells were then washed with wash buffer (20 mM Borate, 150 mM NaCl, 0.2% Tween 20). Samples and standards were added and allowed to incubate at room temperature for 1 hour. Wells again were washed. An antibody conjugated to fluorescein (secondary antibody) was diluted into assay buffer to 2 µg/ml and was then added to the plate and allowed to incubate at room temperature for 1 hour. Wells again were washed. Anti-fluorescein antibody conjugated to alkaline phosphatase, diluted 1/2338 into assay buffer, was added and allowed to incubate at room temperature for 1 hour. Final wash was then performed. Finally substrate (Promega Attophos Product #S1011, Promega Corporation, Madison, Wis.) was added and the plate was read immediately. All additions were 10 ul/well. The plate was washed 3 times between each addition and final wash was 9 times prior to the addition of substrate. Standards were prepared by spiking specific antigen into a normal serum patient pool. Reading was performed using a Tecan Spectrafluor plus (Tecan Inc, Mannedorf, Switzerland) in kinetic mode for 6 read cycles with excitation filter of 430 nm and an emission filter 570 nm emission. Slope of RFU/seconds was determined.

[0383] Final Box and ROC results were analyzed using Analyse-it General+Clinical Laboratory 1.73 (Analyse-it Software Ltd., Leeds England).

3.2 Results

[0384] These experiments identified proteins of particular interest including, but not limited to, OGTA066 (SEQ ID No: 1).

[0385] FIG. 3 shows Box plot data for OGTA066. The vertical axis on this graph is concentration of OGTA066 in ng/ml. These data show decreased concentration of OGTA066 in colorectal cancer samples compared to normal samples, with an almost significant p value, thereby indicating that OGTA066 discriminates well between colorectal cancer and normal, making it a good potential marker for colorectal cancer.

Example 4

Evaluation of Colorectal Cancer Marker Proteins in Multiplex Assay Using Luminex Technology

[0386] Using the following Reference Protocol, OGTA066 was evaluated in a multiplex assay using the Luminex technology.

4.1 Materials and Methods

[0387] Each primary antibody was conjugated to a unique Luminex magnetic microsphere (Mug beads, Luminex Corporation, Austin, Tex.). Mag bead cocktail (50 ul) was added to a 96 black well round bottom Costar plate (Corning Incorporated, Corning N.Y.). Using a 96 well magnetic ring stand, the Mag beads were pulled down for 1 minute and washed with wash/assay buffer (PBS with 1% BSA and 0.02% Tween 20). 50 ul of sample or standard was added along with an additional 50 ul of wash/assay buffer and allowed to incubate on a shaker for 1 hour at room temperature. Plate was placed on magnetic ring stand and allowed to sit for 1 minute. Mag beads were then washed again. Biotin labeled antibody was then added at 50 ul per well with an additional 50 ul of wash/assay buffer and allowed to incubate on a shaker for 1 hour at room temperature. The plate again was placed on a magnetic stand and the Mag beads were washed. Streptavidin-RPE (Prozyme, San Leandro, Calif., Phycolin, Code #PJ31S) was diluted to 1 ug/ml in wash/assay buffer and 50 ul was added to each well along with an additional 50 ul of wash/assay buffer and allowed to incubate on a shaker for 1 hour at room temperature. Final wash was performed and the beads were re-suspended with 100 ul of wash/assay buffer and each well was then read in a Luminex 200 reader using Xponent software 3.0. All reagent dilutions were made in wash/assay buffer. Biotin-antibody varied for each assay to optimal concentration. Initial Mag bead amounts added were approximately 50,000 for each assay. Magnetic beads were allowed 1 minute pull down time prior to each wash. Each wash step was 3 times washed with 100 ul of wash/assay buffer. Assay standard curves were made in a normal donor patient serum pool. Luminex reader and Mag beads were used and prepared according to manufacturer guidelines. Standard curves were calculated using a 5 parameter log-logistic fit and each sample concentration was determined from this curve fit.

[0388] Final Box and ROC results were analyzed using Analyse-it General+Clinical Laboratory 1.73 (Analyse-it Software Ltd., Leeds England).

4.2 Results

[0389] Experiments using 61 normal samples and 65 colorectal cancer samples resulted in further evidence for

OGTA066 (SEQ ID No: 1). FIG. 4 shows ROC curve data for OGTA066 and FIG. 5 shows Box plot data for OGTA066.

[0390] The ROC curves plot sensitivity (true positives) against 1-specificity (false positives). The area under the ROC curve is a measure of the probability that the measured marker level will allow correct identification of a disease or condition. An area of greater than 0.5 indicates that the marker can discriminate between disease and normal. This is the case in the data shown in FIG. 4; OGTA066 has a high area under the curve and a very low p value therefore indicating that OGTA066 is a good potential marker to discriminate between colorectal cancer and normal.

[0391] The vertical axes on the box plots in FIG. 5 is concentration of OGTA066 in ng/ml. FIG. 5 shows lower concentration in serum samples of OGTA066 in colorectal cancer samples than in normal samples. OGTA066 shows good discrimination between colorectal cancer and normal, indicating it may be useful as a marker for colorectal cancer.

[0392] All references referred to in this application, including patent and patent applications, are incorporated herein by reference to the fullest extent possible.

[0393] Throughout the specification and the claims which follow, unless the context requires otherwise, the word 'comprise', and variations such as 'comprises' and 'comprising', will be understood to imply the inclusion of a stated integer, step, group of integers or group of steps but not to the exclusion of any other integer, step, group of integers or group of steps.

[0394] Embodiments of the invention are described herein, which comprise certain elements. The invention also extends to separate embodiments consisting of or consisting essentially of the same elements, and vice versa.

[0395] The application of which this description and claims form part may be used as a basis for priority in respect of any subsequent application. The claims of such subsequent application may be directed to any feature or combination of features described herein. They may take the form of product, composition, process, or use claims and may include, by way of example and without limitation, the following claims:

Sequence Listing

[0396]

Sequence	Sequence ID
MMLHSALGLCLLLVTVSSNLAIATIKKEKRPQTLSRGWGDDI TWVQTYEEGLFYAQSKKPLMVIHHLLEDQYSQALKKVFAQN EEIQEMAQNKFIMLNLMHETTDKNLSPDGQYVPRIMFVDP TVRADIAGRYSNRLYTYEPRDLPPLLIENMKKALRLIQSEL	1
DLPLLIENMK	2
IMFVDP SLTVR	3
KPLMVIHHLLEDQYSQALK	4
NLSPDGQYVPR	5

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 5

<210> SEQ ID NO 1

<211> LENGTH: 166

<212> TYPE: PRT

<213> ORGANISM: Homo Sapiens

<220> FEATURE:

<221> NAME/KEY: SIGNAL

<222> LOCATION: (1)..(22)

<223> OTHER INFORMATION: signal sequence

<220> FEATURE:

<221> NAME/KEY: MISC_FEATURE

<222> LOCATION: (1)..(166)

<223> OTHER INFORMATION: Q8TD06 - Anterior gradient protein 3 homolog
(hAG-3 or BCMP11)

<220> FEATURE:

<221> NAME/KEY: PEPTIDE

<222> LOCATION: (60)..(78)

<223> OTHER INFORMATION: peptide corresponding to SEQ ID NO: 4

<220> FEATURE:

<221> NAME/KEY: PEPTIDE

<222> LOCATION: (108)..(118)

<223> OTHER INFORMATION: peptide corresponding to SEQ ID NO: 5

<220> FEATURE:

<221> NAME/KEY: PEPTIDE

<222> LOCATION: (119)..(129)

<223> OTHER INFORMATION: peptide corresponding to SEQ ID NO: 3

<220> FEATURE:

<221> NAME/KEY: PEPTIDE

<222> LOCATION: (147)..(156)

<223> OTHER INFORMATION: peptide corresponding to SEQ ID NO: 2

<400> SEQUENCE: 1

Met Met Leu His Ser Ala Leu Gly Leu Cys Leu Leu Leu Val Thr Val
1 5 10 15

Ser Ser Asn Leu Ala Ile Ala Ile Lys Lys Glu Lys Arg Pro Pro Gln
20 25 30

Thr Leu Ser Arg Gly Trp Gly Asp Asp Ile Thr Trp Val Gln Thr Tyr
35 40 45

Glu Glu Gly Leu Phe Tyr Ala Gln Lys Ser Lys Lys Pro Leu Met Val
50 55 60

Ile His His Leu Glu Asp Cys Gln Tyr Ser Gln Ala Leu Lys Lys Val
65 70 75 80

Phe Ala Gln Asn Glu Glu Ile Gln Glu Met Ala Gln Asn Lys Phe Ile
85 90 95

Met Leu Asn Leu Met His Glu Thr Thr Asp Lys Asn Leu Ser Pro Asp
100 105 110

Gly Gln Tyr Val Pro Arg Ile Met Phe Val Asp Pro Ser Leu Thr Val
115 120 125

Arg Ala Asp Ile Ala Gly Arg Tyr Ser Asn Arg Leu Tyr Thr Tyr Glu
130 135 140

Pro Arg Asp Leu Pro Leu Leu Ile Glu Asn Met Lys Lys Ala Leu Arg
145 150 155 160

Leu Ile Gln Ser Glu Leu
165

-continued

```
<210> SEQ ID NO 2
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens
```

```
<400> SEQUENCE: 2
```

```
Asp Leu Pro Leu Leu Ile Glu Asn Met Lys
1           5           10
```

```
<210> SEQ ID NO 3
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens
```

```
<400> SEQUENCE: 3
```

```
Ile Met Phe Val Asp Pro Ser Leu Thr Val Arg
1           5           10
```

```
<210> SEQ ID NO 4
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens
```

```
<400> SEQUENCE: 4
```

```
Lys Pro Leu Met Val Ile His His Leu Glu Asp Cys Gln Tyr Ser Gln
1           5           10           15
```

```
Ala Leu Lys
```

```
<210> SEQ ID NO 5
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo Sapiens
```

```
<400> SEQUENCE: 5
```

```
Asn Leu Ser Pro Asp Gly Gln Tyr Val Pro Arg
1           5           10
```

1-82. (canceled)

83. A method comprising administering to a patient having increased levels of OGTA066 as compared to a healthy control a composition comprising an affinity reagent capable of specific binding to OGTA066 as defined in SEQ ID No: 1, or a fragment thereof, and a pharmaceutically acceptable diluent or carrier.

84. The method according to claim **83**, wherein said patient has colorectal cancer, kidney cancer, lung cancer or pancreatic cancer, or has increased likelihood of developing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

85. The method of claim **83**, wherein said affinity reagent comprises a label.

86. The method of claim **84**, wherein said affinity reagent comprises a label.

87. The method according to claim **85**, wherein said label is a detectable label or therapeutic moiety.

88. The method according to claim **86**, wherein said label is a detectable label or therapeutic moiety.

89. The method according to claim **87**, wherein said therapeutic moiety is selected from the group consisting of a cytotoxic moiety and a radioactive isotope.

90. The method according to claim **88**, wherein said therapeutic moiety is selected from the group consisting of a cytotoxic moiety and a radioactive isotope.

91. The method of claim **83**, wherein said affinity reagent is selected from the group consisting of fusion proteins and antibodies.

92. The method of claim **91**, wherein said antibody is a monoclonal antibody, a humanized antibody, a bispecific antibody, a non-fucosylated antibody, an antibody fragment, or an antibody mimetic.

93. The method of claim **83**, wherein said affinity reagent has cytotoxicity against OGTA066 antigen expressing cells in the presence of a human complement or in the presence of human immune effector cells.

94. A method of screening for or diagnosis of, or for monitoring or assessing treatment of colorectal cancer, kidney cancer, lung cancer or pancreatic cancer comprising analyzing a sample from a patient for:

- a. the presence or absence; or
- b. the quantity;

of OGTA066, whereby an increase in the level of OGTA066 as compared to a healthy control is indicative

of the presence of or increased likelihood of developing colorectal cancer, kidney cancer, lung cancer or pancreatic cancer.

95. The method of claim **94** which comprises contacting said sample with one or more anti-OGTA066 affinity reagents capable of specific binding to OGTA066 as defined in SEQ ID No: 1, or a fragment or derivative thereof.

96. The method according to claim **94** wherein said analyzing comprises imaging said sample.

97. The method according to claim **95** wherein said analyzing comprises imaging said sample.

98. The method of claim **96**, wherein said affinity reagent comprises a detectable label.

99. The method of claim **97**, wherein said affinity reagent comprises a detectable label.

100. The method of claim **94**, wherein said method comprises detecting OGTA066 mRNA.

* * * * *