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(54) Title: CANCER

(57) Abstract: The present invention relates to methods for the diagnosis, imaging and treatment of cancer cells. In particular it relates to the use of a tumour suppressor molecule which is apparent at a higher density on cancer cells than normally dividing cells in the diagnosis, imaging, prophylaxis and treatment of cancer.



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CANCER

The present invention relates to methods for the diagnosis, imaging and treatment of cancer cells. In particular it relates to the use of a tumour related molecule which is present at a higher density on cancer cells than normally dividing cells in the
5 diagnosis, imaging, prophylaxis and treatment of cancer.

The chromosomal location of tumour suppressor genes (TSG) has often been first indicated by tumour-associated deletion and loss of heterozygous alleles. The successful identification of these genes has frequently relied on the isolation of
10 candidate TSG from within much smaller homozygously deleted regions, mapping within the larger region of allele loss (1). Various deletion mapping studies have indicated that several distinct regions within the chromosomal 3 loci are involved in the onset and/or progression of lung cancer. Recent studies include a recent extensive study of 151 lung tumour biopsies and cell lines with 28 markers of polymorphic loci
15 (2) on chromosome 3. Using FISH, homozygous deletions at 3p12, 3p14 and 3p21 have been shown to also exist in biopsy material (4).

Despite thorough investigation of genes within these homozygous deletions none has emerged as a "classic" TSG with allele loss surrounding the gene on one
20 chromosome and point mutations in the gene in the remaining homologue. However it is becoming increasingly recognised that tumour suppressor genes may be inactivated by epigenetic mechanisms (6). Several genes on chromosome 3 fall into this category. An isoform of the *123F2/RASSF1* gene and the *FHIT* gene are particularly noteworthy as both have reduced or aberrant expression in lung (and
25 other) tumours and have been shown to suppress tumorigenicity following transfection into tumour cell lines (6,7) Inactivation of the *Fhit* gene in mice results in gastric and sebaceous gland tumour formation in mutant mice challenged intragastrically with carcinogen (8) Therefore, there exists a need in the art to identify other tumour suppressor molecules. Such molecules may be useful in the
30 prophylaxis and/or treatment of tumours.

Cancers may be detected by treating samples of tissues with agents known to bind to defined types of cancer cell. Such detection generally requires removal of tissue samples from a vertebrate. Suitable agents include antibodies and the like. Some cancers can be detected by imaging: radiological methods(e.g. X-rays) are the most familiar. Although valuable, there are problems of selectivity-i.e. other non-malignant nodules are detected and sensitivity-i.e. very small clusters of tumour cells e.g. micrometastases are undetectable.

In an attempt to overcome these problems other methods of obtaining an image of a tumour are being evaluated. For many years tumour -specific antigens were sought but generally have not been found in solid tumours. It is becoming apparent that tumour targets need not be expressed exclusively by tumours to be of value. What is required is that the tumour over-expresses a cell surface antigen compared to surrounding tissue.

Recently, sigma receptor-binding benzamides have been used in the diagnosis and as therapeutic agents for human prostate tumours (27). In this study, it was found that a very high density of sigma receptors is expressed on the androgen-independent human-prostate tumour cell line (DU-145). Both radiolabelled and non-radiolabelled benzamides were shown to bind selectively and with a high affinity to human prostate tumour cells xenografted to nude mice. The three compounds tested all showed a fast clearance from the blood pool and a high uptake and retention in the tumour.. They also showed a dose-dependent inhibition of cell colony formation in two different human prostate cancer cell-lines. There remains a need in the art, therefore to identify further cell surface molecules the cell surface density of which is different when normally dividing cells are compared with cancer cells. Identification of such molecules will be of use in the imaging and/or diagnosis of cancer.

Previously, The present inventors have described a large homozygous deletion at 3p12-13 in an SCLC line, U2020. Following construction of a physical map of this region, CpG island mapping was used to identify the gene location (9) . One such gene, named *DUTTI* (deleted in U2020) was found to map within smaller

homozygous deletions in two other tumour cell lines (9). The *DUTT1/ROBO1* gene is widely expressed in mammals and codes for a receptor with a domain structure of the NCAM family (13). Several lung tumour cell lines including NIH-H219X have previously been shown to possess one or more deletions within the gene encoding
5 DUTT1, therefore implicating DUTT1 in lung cancer.

The present inventors set out to overcome the problems of the prior art. In particular, they set out to identify one or more molecules which play a role in the onset or progression of lung tumour formation. In addition they set out to establish a method
10 for the imaging, diagnosis, prophylaxis and/or treatment of cancer cells.

Summary of the invention

The present inventors have surprisingly found that the level of the tumour suppressor protein DUTT1 is high in cancer cells, including carcinoma insitu (pre-invasive cancer
15 cells), and is low on non-cancerous epithelium, for instance bronchial epithelium. In addition, negligible protein is detected on epithelial stem cells. This is a surprising finding and contrary to expectations as the present inventors have shown, as described herein (Figures 1 to 4), that decreasing the functional activity of DUTT1 in cells, particularly cells comprising the lung causes the appearance of bronchial epithelial
20 hyperplasia in lung cells.

In a first aspect, the present invention provides a method for the early detection of cancer in a population of cells comprising the steps of:

- (1) providing a population of cells
- 25 (2) assaying the cell population for an increased level of DUTT1 in any one or more of those cells as compared with normally dividing cells.

Thus in a second aspect, the present invention provides a method for the early detection of cancer in a population of cells comprising the steps of :

- 30 (1) providing a population of cells
- (2) assaying those cells and establishing a reference level of DUTT1,
- (3) obtaining a population of cells for diagnosis,

- (4) assaying the cell population of step 3, for an increased level of DUTT1 in any one or more of those cells when compared with the reference level of DUTT1.

In a further aspect, the present invention provides a method for the diagnosis of cancer
5 in a population of cells comprising the steps of:

- (1) providing a population of cells
- (2) assaying the cell population for an increased level of DUTT1 in any one or more of those cells as compared with normally dividing cells.

10 Thus in a further aspect still, the present invention provides a method for the diagnosis of cancer in a population of cells comprising the steps of :

- (1) providing a population of cells
- (2) assaying those cells and establishing a reference level of DUTT1,
- (3) obtaining a population of cells for diagnosis,
- 15 (4) assaying the cell population of step 3, for an increased level of DUTT1 in any one or more of those cells when compared with the reference level of DUTT1.

Common characteristics of 'cancer' as herein defined include the ability of a cell to undergo endless replication, loss of contact inhibition, invasiveness and the ability to
20 metastasize. That is, when the cell divides in an uncontrollable way and can not recognise its own natural boundary, the cancer cells obtain the ability to spread to other areas of the body. Mutations within the nucleic acid of one or more cells are involved in the onset of cancer. Often, more than one nucleic acid mutation or other aberrant cellular event is required for the development of tumours (bundles of
25 aberrantly dividing cells), that is tumour formation is a multi-signal event. In the context of the present invention cancer cells include any cells which exhibit any one or more of the following features aberrant cell division, aberrant contact inhibition, aberrant cell differentiation as compared with cells behaving normally within their native environment, the ability of the cell to invade tissues, and the ability to
30 metastasise. The definition of 'cancer cells' in the context of the present invention, therefore includes within its scope tumour cells and also cells prior to the formation of tumours in so far as they possess one or more of the requisite characteristics listed

above. In addition the term cancer cells according to the present invention includes metastatic cells.

The method of the present invention is suitable for the diagnosis and/or detection of many forms of cancer. In particular, the cancer may be one or more selected from the group consisting of: epithelial cancer, sarcoma and lymphoma. In a preferred embodiment of the above two aspects of the invention, the cancer is lung cancer, advantageously it is human lung cancer. Advantageously, the cells for diagnosis include bronchial epithelial cells and/or bronchial hyperplasia of epithelium, and/or cells derived from lymph nodes Preferably, the method is an in vitro method.

In the context of the present invention, the term the 'early detection' of cancer means the detection of cancer prior to the onset of one or more clinical signs of cancer in a patient. Clinical signs of cancer will be known to those skilled in the art and includes the formation of tumours and metastases. The early detection of cancer

One skilled in the art will appreciate that the cell population of steps (1) and (3) may be the same population. In practice steps (1) to (4) may be performed simultaneously, or separately. Alternatively, several steps may be performed simultaneously, and others separately.

A reference level of DUTT1 may be established using one or more agents selected from the group consisting of: anti-DUTT1 antibodies, DUTT1 binding peptides and small molecules which bind to DUTT1. Advantageously, DUTT1 antibodies are used. Suitable methods for measuring DUTT1 levels using these agents will be familiar to one skilled in the art and are described herein.

In addition or alternatively DUTT1 binding agents may be bound to a population of cells, and a reference level of DUTT1 established by directly comparing cancerous and non-cancerous cells within the same cell population. This has the advantage that protein levels in cancer cells and surrounding normally dividing cells can be compared easily and simultaneous, and that quantitative measurements of DUTT1 levels do not

need to be made. Cells binding an increased level of DUTT1 binding agent, compared with the normal cells will be easily distinguishable.

Advantageously, the DUTT1 binding agents will comprise or have associated with them means for their detection. Suitable means may be naturally occurring or synthetic molecules and will be familiar to those skilled in the art, and are described herein.

In a further aspect, the present invention provides, the use of a DUTT1 binding agent in the diagnosis of cancer.

10

The use according to the present invention is suitable for the diagnosis of many forms of cancer. In particular, the cancer may be one or more selected from the group consisting of: epithelial cancer, sarcoma and lymphoma. In a preferred embodiment of the above two aspects of the invention, the cancer is lung cancer, advantageously it is human lung cancer. Advantageously, the cells for diagnosis include bronchial epithelial cells and/or bronchial hyperplasia of epithelium, and/or cells derived from lymph nodes Preferably, the method is an in vitro method.

Suitable DUTT1 binding agents for diagnosis are as herein described,. Preferably the DUTT1 binding agent is an antibody raised against DUTT1.

20

Due to the surprising finding that that the level of the tumour suppressor DUTT1 is higher on cancer cells including carcinoma in situ cells, than on non-cancer cells, then DUTT1 binding agents can be used as a method of imaging or visualising cancer cells.

25

Thus in a further aspect, the present invention provides a method for the selective labelling of cancer cells comprising the step of treating one or more cancer cells with a DUTT1 binding agent.

In yet a further aspect, the present invention provides the use of a DUTT1 binding agent in the selective labelling of cancer cells.

30

Cells suitable for selective labelling include any cell which expresses DUTT1, preferably at high levels. Suitable cells include epithelial cancer cells, sarcoma cells and lymphoma cells. In a preferred embodiment of the invention, the cells are lung cancer cells, preferably human lung cancer cells. Suitably, the cancer cells are
5 metastatic cells and/or primary cancer cells.

Advantageously, the method is for the selective labelling of cancer cells selected from the group consisting of: human cancer cells, human epithelial cancer cells, human sarcoma cells, human haematopoietic cells.

10

Suitable DUTT1 binding agents are as described herein.

The term 'labelling' in the context of the present invention means the selective binding of an agent to cells, in this case to cancer cells and not to normally dividing cells.

15 Advantageously the labelling agent comprises or has associated with it means permitting the detection of the label. Suitable means include fluorescent, phosphorescent, or radio-active agents as herein described. One skilled in the art will appreciate that this list is not intended to be exhaustive. Suitable means for detection of the labelled cells will vary according to whether the labelled cells are to be detected in
20 vitro or in vivo. Suitable in vitro methods include but are not limited to autoradiography and fluorescence or phosphorescence detection. Suitable, in vivo methods include but are not limited to nuclear magnetic resonance, autoradiography and so on. Those skilled will be aware of other suitable methods. In this way the method of this aspect of the present invention may be used for the in vivo imaging of
25 cancer cells, preferably metastatic cancer cells. Advantageously, the method is used for the imaging of human cancer cells. Most preferably, the method is used for the imaging of human cancer cells, which may be primary cancer cells or metastatic cells within the body of a patient.

30 Suitable DUTT1 binding agents include any one or more selected from the group consisting of the following: anti-DUTT1 antibodies, DUTT1 binding peptides and small molecules which bind to DUTT1. Advantageously, DUTT1 antibodies are used.

In a further aspect, the present invention provides a method for the imaging of cancer cells comprising the step of treating one or more cancer cells with one or more DUTT1 binding agent/s wherein the DUTT1 binding agent further comprises detection means.

5

In the context of the present invention the term 'imaging' refers to the generation of a 2 dimensional or 3 dimensional picture/image of the cancer cells. Where the imaging is performed within an in vivo environment, it may allow the relative position of the cancer cells within the in vivo environment, preferably the human body to be established. Due to the high density of DUTT1 found on lung cancer cells including pre-invasive cancer cells, a high signal to noise ratio can be achieved, allowing high sensitivity imaging. Such a high signal to noise ratio is particularly important in the detection of small bundles of cancer cells, that is metastatic cells. One skilled in the art will appreciate though that this method is also of use in the imaging of primary cancer cells.

15

Preferably the method is for the in vivo imaging of cancer cells. More preferably it is for the imaging of human cancer cells. Advantageously, the method is for the imaging of cancer cells selected from the group consisting of: human epithelial cancer cells, human sarcoma cells, human haematopoietic cancer cells.

20

In a further aspect still, the present invention provides a composition comprising DUTT1, or a binding agent thereof.

25 In a further aspect still, the present invention provides a method for the internalisation of one or more DUTT1 binding agents comprising the step of treating a cell with one or more DUTT1 binding agents.

30 In the context of the present invention, the term 'treating' refers to the process of bringing one or more cells into contact with a DUTT1 binding agent. Suitable methods for 'treating' cells will depend upon whether the procedure is carried out in vivo or in

vitro and are described herein. In a preferred embodiment of this aspect of the invention, the method occurs in vivo.

As herein defined, the term 'internalisation' means bringing one or more molecules
5 into the interior of the cell. Advantageously, it describes bringing one or more molecules into the cytoplasm of a cell. In the present case at least one molecule is a DUTT1 binding agent. Suitable DUTT1 binding agents are herein described.

Cells suitable for treatment with a DUTT1 binding agent according to the method of
10 the present invention includes any cell which expresses DUTT1. Suitable cells include epithelial cells. Those skilled in the art will be aware of other suitable cell types.

Suitable DUTT1 binding agents are herein described. Advantageously, the DUTT1
15 binding agent is a antibody raised against DUTT1. Preferably, it is a monoclonal antibody.

In a final aspect, the present invention provides the use of DUTT1, and/or a binding
agent thereof in the preparation of a medicament for the prophylaxis or treatment of
cancer.

20 In one embodiment of this aspect of the invention, the use is in the treatment of lung cancer. In a further embodiment of this aspect of the invention, the use is in the treatment of epithelial cancer. In a further embodiment still, the use is in the treatment of sarcoma. In a further preferred embodiment, the use is in the treatment of
25 lymphoma.

Brief description of the figures

Fig. 1. Targeted mutation of the *Dutt1/Robo1* gene. **A**, the genomic structure of the targeted locus and the targeting construct and mutant allele. The filled box represents
30 exon 2. Fragments a and b are the hybridisation probes used in (B) below. The outer dashed lines indicate the fragment used for homologous recombination. E EcoRI, S SacI, B BamHI. **B**, Following transfection of the targeting construct into ES cells,

SacI digested DNA was prepared from G418 resistant ES(+/-) and control ES cells(+/+) and analysed by Southern blot analysis. The filter was hybridised to probe [a] which flanks the site of homologous recombination at the 3' end, and with probe [b] external to the homologous integration, not shown. Two clones showing correct homologous recombination (i.e. both wild type, 6.9kb and mutant, 7.3 kb alleles) were injected into blastocysts and the resulting chimaeras bred with C57BL/6 mice to obtain transmission of the mutant allele in the germ-line, results not shown. C, Finally mice with germ-line transmission, *Dutt1/Robo1*^{+/-}, were inter-crossed to obtain offspring which were homozygous (-/-) for the targeted mutation. SacI digested tail DNA from offspring from this inter-cross was screened by Southern blotting with probe [a].

Fig. 2. *Dutt1/Robo1* protein analysis (24). **A**, Western blotting analysis of protein isolated from the organs shown from wild type (+/+) and *Dutt1/Robo1*^{-/-} (-/-) day 15 embryos using antiserum raised against a C-terminal DUTT1/ROBO1 peptide. **B**, phase contrast image of a 4 micrometre transverse section of paraformaldehyde fixed normal new-born lung at x200 magnification. **C**, detection by immunohistochemistry of *Dutt1/Robo1* protein using anti-peptide antiserum in (A) in bronchial epithelium with Cy-3-TSA amplification system (NEN Life Sciences) applied to the same section as in (B) x200 magnification.

Fig. 3. Gross morphological phenotypes of wild-type and *Dutt1/Robo1*^{-/-} mice. A wild type mouse and its lungs shown below (+/+) compared to a *Dutt1/Robo1*^{-/-} mouse and its lungs shown below (-/-).

Fig. 4. Histological analysis of lungs of *Dutt1/Robo1*^{-/-} mice and wild-type littermates. Paraformaldehyde fixed 4 micrometre sections of lung tissue were H&E stained and photographed. **A**, wild-type lung, embryo day E15.5 x 400 magnification, **B**, *Dutt1/Robo1*^{-/-} lung, embryo day 15.5, x 400 magnification, **C**, wild-type newborn lung, x 100 magnification, **D**, *Dutt1/Robo1*^{-/-} newborn lung x 100 magnification (arrow indicates bronchi), **E**, wild-type adult lung x 100 magnification, **F**, *Dutt1/Robo1*^{-/-} adult lung. x 100 magnification, **G**, wild-type adult

lung at x 400 magnification, **H**, *Dutt1/Robo1*^{-/-} adult lung, at x 400 magnification, **I**, *Dutt1/Robo1*^{-/-} adult lung, at x 400 magnification, focal dysplasia indicated by arrow.

Populations of mice were established from two independent ES clones: both gave indistinguishable abnormal lung pathology.

5

Fig 5. Detection by immunohistochemistry of DUTT1/ROBO1 protein using anti-peptide antibody in a section of formalin fixed human squamous cell carcinoma of the lung .

10 **Fig 6.** Detection by immunohistochemistry of DUTT1/ROBO1 protein using anti-peptide antibody in a section of formalin fixed human normal bronchial epithelium

Definitions

15 Common characteristics of ‘**cancer**’ include the ability of the cancer cell to undergo endless replication, loss of contact inhibition, invasiveness and the ability to metastasise. That is, when the cell divides in an uncontrollable way and can not recognise its own natural boundary, the cancer cells obtain the ability to spread to other areas of the body. Mutations within the

20 nucleic acid of one or more cells are involved in the onset of cancer. Often, more than one nucleic acid mutation or other aberrant cellular event is required for the development of tumours (bundles of aberrantly dividing cells), that is tumour formation is a multi-signal event. In the context of the present invention cancer cells include any cells which exhibit any one or

25 more of the following features aberrant cell division, aberrant contact inhibition, aberrant cell differentiation as compared with cells behaving normally within their native environment, the ability of the cell to invade tissues, and the ability to metastasise. The definition of ‘cancer cells’ in the context of the present invention, therefore includes within its scope tumour

30 cells and also cells prior to the formation of tumours in so far as they possess one or more of the requisite characteristics listed above. In

addition the term cancer cells according to the present invention includes metastatic cells.

A '**tumour suppressor molecule**' is a molecule one function of which is to suppress tumourigenesis. Certain cancers have been found to be associated with mutant suppressor genes for example p53 and RB. However as indicated above often more than one abherent cell component or signal is required to initiate and/or to cause the progression of cancer.

'Bronchial epithelial hyperplasia'. The airway of the lung in descending order of size are bronchi, bronchioles and alveoli. Bronchial refers to the first two. . The lung consists of mesenchymal cells and epithelial cells which line the airways. **Hyperplasia** means over growth. The epithelium is normally a sheet of cells one layer thick but when it becomes hyperplastic it thickens and becomes disorderly. Bronchial epithelial hyperplasia. as herein defined is thus disordered cell morphology in the cell layer lining the main airways of the lung. This form of hyperplasia is associated with the early stages of lung (and other) cancers and the term cancer as herein defined includes within its scope bronchial epithelial hyperplasia.

'Specific labelling of a cell' in the context of the present invention means the selective/specific binding of a labelling agent to a cell. That is, that when a labelling agent is exposed to a cell population, only those cells showing certain characteristics will bind to the labelling agent. Generally, the characteristics include the presence of certain cell surface features, for example the presence of a surface antigen which binds to the labelling agent.

An '**Antibody**' (for example IgG, IgM, IgA, IgD or IgE) or fragment (such as a FAb, F(Ab')₂, Fv, disulphide linked Fv, scFv, diabody) whether derived from any species naturally producing an antibody, or created by recombinant DNA technology; whether isolated from serum, B-cells, hybridomas, transfectomas, yeast or bacteria).

DETAILED DESCRIPTION OF THE INVENTION

General Techniques

5 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular genetics, nucleic acid chemistry, hybridisation techniques and biochemistry). Standard techniques are used for molecular, genetic and biochemical methods (see generally, Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d
10 ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999) 4th Ed, John Wiley & Sons, Inc. which are incorporated herein by reference) and chemical methods. In addition Harlow & Lane., A Laboratory Manual Cold Spring Harbor, N.Y, is referred to for standard Immunological Techniques.

15

Method for the diagnosis of cancer in a vertebrate

In a first aspect, the present invention provides a method for the early diagnosis of cancer in a population of cells comprising the steps of:

(1)providing a population of cells

20 (2)assaying the cell population for an increased level of DUTT1 in any one or more of those cells as compared with normally dividing cells.

In a second aspect, the present invention provides a method for the early diagnosis of cancer in a population of cells comprising the steps of :

25 (1)providing a population of cells

(2)assaying those cells and establishing a reference level of DUTT1,

(3)obtaining a population of cells for diagnosis,

(4) assaying the cell population of step 3, for an increased level of DUTT1 in any one or more of those cells when compared with the reference level of DUTT1.

30

In a further aspect, the present invention provides a method for the diagnosis of cancer in a population of cells comprising the steps of:

- (1)providing a population of cells
- (2)assaying the cell population for an increased level of DUTT1 in any one or more of those cells as compared with normally dividing cells.

5 In a further aspect still, the present invention provides a method for the diagnosis of cancer in a population of cells comprising the steps of :

- (1)providing a population of cells
- (2)assaying those cells and establishing a reference level of DUTT1,
- (3)obtaining a population of cells for diagnosis,
- 10 (4)assaying the cell population of step 3, for an increased level of DUTT1 in any one or more of those cells when compared with the reference level of DUTT1.

(A) Cells for diagnosis.

15 Cell samples are obtained from a vertebrate for treatment using methods familiar to those skilled in the art. In the case of small vertebrates the animals may be sacrificed, and the tissue for diagnosis extracted. Tissue slices may be prepared using methods familiar to those skilled in the art.

20 Alternatively, cells for diagnosis may be obtained by performing a biopsy (removal of a small amount of tissue from a vertebrate, which is preferably alive).

(B) Establishing a reference level of DUTT1

Techniques such as Northern blotting and Western blotting of cell extracts followed by
25 hybridisation with agents which bind to DUTT1 may be used in the measurement of DUTT1 levels. Agents which bind to DUTTI include monoclonal and polyclonal antibodies, DUTT1 binding peptides and small molecules which bind to DUTT1. In addition or alternatively DUTT1 binding agents may be bound to a population of cells, and a reference level of DUTT1 established by directly comparing cancerous and non-
30 cancerous cells within the same cell population. This has the advantage that protein levels in cancer cells and surrounding normally dividing cells can be compared easily

and simultaneous, and that quantitative measurements of DUTT1 levels do not need to be made..

(Bi)DUTT1 binding agents

5 (a) Antibodies and peptides

Antibodies raised against DUTT1, and DUTT1 peptides may be prepared using standard laboratory techniques. Either recombinant proteins or those derived from natural sources can be used to generate antibodies. For example, the protein (or “immunogen”) is administered to challenge a mammal such as a monkey, goat, rabbit
10 or mouse. The resulting antibodies can be collected as polyclonal sera, or antibody-producing cells from the challenged animal can be immortalized (e.g. by fusion with an immortalizing fusion partner to produce a hybridoma), which cells then produce monoclonal antibodies.

15 Polyclonal antibodies

The antigen protein is either used alone or conjugated to a conventional carrier in order to increase its immunogenicity, and an antiserum to the peptide-carrier conjugate is raised in an animal, as described above. Coupling of a peptide to a carrier protein and immunizations may be performed as described (Dymecki et al. (1992) J.
20 Biol. Chem., **267**: 4815). The serum is titrated against protein antigen by ELISA or alternatively by dot or spot blotting (Boersma and Van Leeuwen (1994) J. Neurosci. Methods, **51**: 317). The serum is shown to react strongly with the appropriate peptides by ELISA, for example, following the procedures of Green et al. (1982) Cell, **28**: 477.

25 Monoclonal antibodies

Techniques for preparing monoclonal antibodies are well known, and monoclonal antibodies may be prepared using any candidate antigen, preferably bound to a carrier, as described by Arnheiter et al. (1981) Nature, **294**, 278. Monoclonal antibodies are typically obtained from hybridoma tissue cultures or from ascites fluid
30 obtained from animals into which the hybridoma tissue was introduced. Nevertheless, monoclonal antibodies may be described as being “raised against” or “induced by” a protein.

After being raised, monoclonal antibodies are tested for function and specificity by any of a number of means. Similar procedures can also be used to test recombinant antibodies produced by phage display or other in vitro selection technologies.

- 5 Monoclonal antibody-producing hybridomas (or polyclonal sera) can be screened for antibody binding to the immunogen, as well. Particularly preferred immunological tests include enzyme-linked immunoassays (ELISA), immunoblotting and immunoprecipitation (see Voller, (1978) Diagnostic Horizons, 2: 1, Microbiological Associates Quarterly Publication, Walkersville, MD; Voller et al. (1978) J. Clin. Pathol., 31: 507; U.S. Reissue Pat. No. 31,006; UK Patent 2,019,408; Butler (1981) Methods Enzymol., 73: 482; Maggio, E. (ed.), (1980) Enzyme Immunoassay, CRC Press, Boca Raton, FL) or radioimmunoassays (RIA) (Weintraub, B., Principles of radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March 1986, pp. 1-5, 46-49 and 68-78), all to detect binding of the antibody to the immunogen against which it was immunogen must be labeled to facilitate such detection. Techniques for labelling antibody molecules are well known to those skilled in the art (see Harlow and Lane (1989) Antibodies, Cold Spring Harbor Laboratory, pp. 1-726.).

20 (Bii) RNA (Northern) blotting and Western (antibody) blotting

Northern and Western blotting may be performed using methods familiar to those skilled in the art and detailed in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2d ed. (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. and Ausubel et al., Short Protocols in Molecular Biology (1999).

25

(C) Assaying tissue samples for binding to DUTT1 binding agents

Tissue samples may be prepared using methods familiar to those skilled in the art and DUTT1 detected using any of the reagents referred to above. An example of a typical protocol is detailed below:

30

Tissues or whole embryos are fixed in 4% paraformaldehyde in PBS buffer for at least 24h, paraffin embedded and processed to give 4 mm sections. Sections were

deparaffinised and endogenous peroxide quenched. After blocking, Dutt1/Robo1 was detected using the polyclonal antisera raised against DUTT1 followed by donkey biotin-conjugated anti-rabbit secondary antibody (SantaCruz USA) and amplification with the Vectastain ABC kit (Vector Laboratories USA) as per the manufacturers protocol. Positive staining was detected using nickel enhanced diaminobenzidine tetrahydrochloride (DAB) and counterstained with Fast Red. Slides were mounted with Vectashield-mounting medium. Staining was blocked by preincubation of the immunising peptide with the antisera. Sections were stained with haematoxylin and eosin (H&E).

The functional portion of the DUTT1 binding agent, when the DUTT1 binding agent, is used for diagnosis, usually comprises and may consist of a radioactive atom for scintigraphic studies, for example technetium 99m (^{99m}Tc) or iodine-123 (^{123}I).

15 **Selective labelling of cancer cells**

In a further aspect, the present invention provides a method for the selective labelling of cancer cells comprising the step of treating one or more cancer cells with a DUTT1 binding agent.

20 The term 'labelling' according to the present invention means the selective binding of an agent to cells, in this case to cancer cells and not to normally dividing cells. Advantageously the labelling agent comprises or has associated with it means permitting the detection of the label. Suitable means include fluorescent, phosphorescent, or radio-active agents, or radioopaque molecules or agents, such as
25 metal particles, which are readily visualised within an embryo or a cell mass.

Suitable cancer cell, particularly lung cancer cell labelling agents include antibodies raised against DUTT1 as herein defined, DUTT1 binding peptides, and/or small molecule agonists which mimic the binding of the natural ligand to the receptor.

30

Particularly indicated are immunostaining and FACS techniques. Suitable fluorophores are known in the art, and include chemical fluorophores and fluorescent polypeptides,

attached to immunoglobulin molecules by incorporating binding sites therefor into the immunoglobulin molecule during the synthesis thereof.

Preferably, the fluorophore is a fluorescent protein, which is advantageously GFP or a mutant thereof. GFP and its mutants may be synthesised together with the immunoglobulin or target molecule by expression therewith as a fusion polypeptide, according to methods well known in the art. For example, a transcription unit may be constructed as an in-frame fusion of the desired GFP and the immunoglobulin or target, and inserted into a vector as described above, using conventional PCR cloning and ligation techniques.

Antibodies may be labelled with any agent capable of generating a signal. The signal may be any detectable signal, such as the induction of the expression of a detectable gene product. Examples of detectable gene products include bioluminescent polypeptides, such as luciferase and GFP, polypeptides detectable by specific assays, such as β -galactosidase and CAT, and polypeptides which modulate the growth characteristics of the host cell, such as enzymes required for metabolism such as HIS3, or antibiotic resistance genes such as G418. In a preferred aspect of the invention, the signal is detectable at the cell surface. For example, the signal may be a luminescent or fluorescent signal, which is detectable from outside the cell and allows cell sorting by FACS or other optical sorting techniques.

Preferred is the use of optical immunosensor technology, based on optical detection of fluorescently-labelled antibodies. Immunosensors are biochemical detectors comprising an antigen or antibody species coupled to a signal transducer which detects the binding of the complementary species (Rabbany *et al.*, 1994 *Crit Rev Biomed Eng* 22:307-346; Morgan *et al.*, 1996 *Clin Chem* 42:193-209). Examples of such complementary species include the antigen Zif 268 and the anti-Zif 268 antibody. Immunosensors produce a quantitative measure of the amount of antibody, antigen or hapten present in a complex sample such as serum or whole blood (Robinson 1991 *Biosens Bioelectron* 6:183-191). The sensitivity of immunosensors makes them ideal

for situations requiring speed and accuracy (Rabbany *et al.*, 1994 *Crit Rev Biomed Eng* 22:307-346).

Detection techniques employed by immunosensors include electrochemical,
5 piezoelectric or optical detection of the immunointeraction (Ghindilis *et al.*, 1998 *Biosens Bioelectron* 1:113-131). An indirect immunosensor uses a separate labelled species that is detected after binding by, for example, fluorescence or luminescence (Morgan *et al.*, 1996 *Clin Chem* 42:193-209). Direct immunosensors detect the
10 binding by a change in potential difference, current, resistance, mass, heat or optical properties (Morgan *et al.*, 1996 *Clin Chem* 42:193-209). Indirect immunosensors may encounter fewer problems due to non-specific binding (Attridge *et al.*, 1991 *Biosens Bioelectron* 6:201-214; Morgan *et al.*, 1996 *Clin Chem* 42:193-209). such as GFP and mutants thereof (see WO 97/28261).

15 Imaging of cancer cells

A method for the imaging of cancer cells comprising the step of treating one or more cancer cells with one or more DUTT1 binding agent/s wherein the DUTT1 binding agent further comprises detection means.

20 Suitable detection means as herein defined include molecules/agents which can be readily detected when associated with, or form a component of the specific labelling agent as herein defined, when present within an in vivo environment, preferably the human body.

25 (1) Emission of radioactive particles

Examples of suitable radioactive agents/molecules include technetium 99m (^{99m}Tc) or iodine-123 (^{123}I). Tumours can then readily be visualised by detecting the emission of radioactive particles using methods known to those skilled in the art.

30 (2) Nuclear Magnetic resonance (NMR)/magnetic resonance imaging (MRI)

Detection molecules/agents such as iodine-123, iodine-313, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron allow visualisation

of cancer cells using NMR. This has the advantage that a whole body scanning can be performed.

(3) Positron Emission Tomography (PET)

- 5 This method differs from standard nuclear magnetic resonance imaging in that short lived isotopes are used which emit positrons. The positrons then interact with normal tissue electrons to produce pairs of 511KeV photons which can then be detected using a very sensitive positron emission tomographic camera.

Suitable detection means for use in PET include ^{11}C methionine and FDG.

- 10 Descriptions of procedures and protocols for using PET are familiar to those skilled in the art..

Further uses of DUTT1, and/or DUTT1 binding agent/s.

- 15 In a final aspect, the present invention provides the use of DUTT1, and/or a binding agent thereof in the preparation of a medicament for the prophylaxis or treatment of cancer.

Therapeutic and prophylactic uses of DUTT1 and/or binding agents thereof involve the administration of the above to a recipient mammal, such as a human.

20

- Substantially pure DUTT1 and/or binding agents thereof of at least 90 to 95% homogeneity are preferred for administration to a mammal, and 98 to 99% or more homogeneity is most preferred for pharmaceutical uses, especially when the mammal is a human. Once purified, partially or to homogeneity as desired, the DUTT1 and/or
25 binding agents thereof may be used diagnostically or therapeutically (including extracorporeally) or in developing and performing assay procedures using methods known to those skilled in the art.

- DUTT1 and binding agents thereof, may be effective in treating cancer related
30 diseases. The present invention includes the method of treating cancer related disease with an effective amount of DUTT1 or DUTT1 binding agents, according to the present invention. The DUTT1 and DUTT1 binding agents of the present invention

can be provided as isolated and substantially purified proteins and protein fragments in pharmaceutically acceptable compositions using formulation methods known to those of ordinary skill in the art. These compositions can be administered by standard routes. These include but are not limited to: oral, rectal, ophthalmic (including
5 intravitreal or intracameral), nasal, topical (including buccal and sublingual), intrauterine, vaginal or parenteral (including subcutaneous, intraperitoneal, intramuscular, intravenous, intradermal, intracranial, intratracheal, and epidural) transdermal, intraperitoneal, intracranial, intracerebroventricular, intracerebral, intravaginal, intrauterine, or parenteral (e.g., intravenous, intraspinal, subcutaneous or
10 intramuscular) routes.

The DUTT1 and DUTT1 binding agents may conveniently be presented in unit dosage form and may be prepared by conventional pharmaceutical techniques. Such techniques include the step of bringing into association the active ingredient and the
15 pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

20 In addition, the DUTT1 and DUTT1 binding agents of the present invention may be incorporated into biodegradable polymers allowing for sustained release of the compound, the polymers being implanted in the vicinity of where drug delivery is desired, for example, at the site of a tumor or implanted so that the DUTT1 binding agent or fragment is slowly released systemically. The biodegradable polymers and
25 their use are described, for example, in detail in Brem *et al* (J. Neurosurg 1991 74:441-446). Osmotic minipumps may also be used to provide controlled delivery of high concentrations of DUTT1 or binding agents thereof, including fragments thereof through cannulae to the site of interest, such as directly into a metastatic growth or into the vascular supply to that tumor.

30

The DUTT1 and DUTT1 binding agents of the present invention may be linked to cytotoxic agents which are infused in a manner designed to maximize delivery to the

desired location. For example, ricin-linked high affinity DUTT1, and/or binding agents thereof are delivered through a cannula into vessels supplying the target site or directly into the target. Such agents are also delivered in a controlled manner through osmotic pumps coupled to infusion cannulae.

5

Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, as herein above recited, or an appropriate fraction thereof, of the administered ingredient. It should be understood that in addition to the ingredients, particularly mentioned above, the formulations of the present invention may include
10 other agents conventional in the art having regard to the type of formulation in question.

DUTT1 or binding agents thereof may be administered in any suitable way, usually parenterally, for example intravenously or intraperitoneally, in standard sterile, non-
15 pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered intravenously). Once DUTT1 or binding agents has bound to the target cells and been cleared from the bloodstream (if necessary), which typically takes a day or so, the pro-drug is administered, usually as a single infused dose, or the tumour is imaged. If needed, because DUTT1 or the DUTT1 binding agent may be
20 immunogenic, cyclosporin or some other immunosuppressant can be administered to provide a longer period for treatment but usually this will not be necessary.

The timing between administrations of DUTT1 binding agents and/or fragments thereof may be optimised in a non-inventive way since tumour/normal tissue ratios of
25 conjugate (at least following intravenous delivery) are highest after about 4-6 days, whereas at this time the absolute amount of DUTT1 binding agents, or fragments of bound to the tumour, in terms of percent of injected dose per gram, is lower than at earlier times.

30 Therefore, the optimum interval between administration of DUTT1 binding agent and/or fragments thereof will be a compromise between peak tumour concentration of and the best distribution ratio between tumour and normal tissues. The dosage of the

DUTT1 binding agent or fragment thereof will be chosen by the physician according to the usual criteria. At least in the case of methods employing a targeted enzyme such as β -glucosidase and intravenous amygdalin as a toxic pro-drug, 1 to 50 daily doses of 0.1 to 10.0 grams per square metre of body surface area, preferably 1.0-5.0 g/m² are likely to be appropriate. For oral therapy, three doses per day of 0.05 to 10.0g, preferably 1.0-5.0g, for one to fifty days may be appropriate. The dosage of DUTT1 binding agent or fragment thereof will similarly be chosen according to normal criteria, particularly with reference to the type, stage and location of the tumour and the weight of the patient. The duration of treatment will depend in part upon the rapidity and extent of any immune reaction to the DUTT1 binding agent, or fragment thereof.

When used in a compound for selective destruction of the tumour, the functional portion of DUTT1 binding agent, or fragment thereof may comprise a highly radioactive atom, such as iodine-131, rhenium-186, rhenium-188, yttrium-90 or lead-212, which emits enough energy to destroy neighbouring cells, or a cytotoxic chemical compound such as methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), daunorubicin or other intercalating agents.

The radio- or other detection agents may be incorporated in the DUTT1 binding agent and/or fragments thereof in known ways. For example, a DUTT1 binding peptide may be biosynthesised or may be synthesised by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as ^{99m}Tc, ¹²³I, ¹⁸⁶Rh, ¹⁸⁸Rh and ¹¹¹In can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker *et al* (1978) Biochem. Biophys. Res. Commun. 80: 49-57) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

Pharmaceutical compositions comprising an effective amount of DUTT1 binding agent and/or a fragment thereof can be used in the prophylaxis, suppression or treatment of cancer related disorders. Such disorders include but not limited to: solid tumours;

blood born tumours such as leukemias; tumor metastasis; benign tumours, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; rheumatoid arthritis; psoriasis; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis; Osler-Webber Syndrome; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; wound granulation; corornay collaterals; cerebral collaterals; arteriovenous malformations; ischeniic limb angiogenesis; neovascular glaucoma; retrolental fibroplasia; diabetic neovascularization; heliobacter related diseases, fractures, vasculogenesis, hematopoiesis, ovulation, menstruation and placentation.

In the instant application, the term "prevention" involves administration of the protective composition prior to the induction of the disease. "Suppression" refers to administration of the composition after an inductive event, but prior to the clinical appearance of the disease. "Treatment" involves administration of the protective composition after disease symptoms become manifest.

The invention is further described, for the purposes of illustration only, in the following examples which are in no way limiting of the invention.

EXAMPLES

Example 1

DNA sequencing and contig assembly of mouse *Dutt1/Robo1* cDNA clones. A mouse brain cDNA library, purchased from Stratagene (La Jolla, CA) and a mouse 5 13.5 day embryo cDNA library, purchased from Life Technology were screened with human *DUTT1/ROBO1* cDNA clones. Positive cDNA clones were sequenced and overlapped to generate a contig map that was entered into the Genbank data base. All sequencing reactions were performed using the Sanger dideoxy chain termination method using the Sequenase version 2 kit (United States Biochemical, USB). The 10 sequence of the cDNA clones was independently confirmed (Oswell Research Products Ltd., Lab 5005, Southampton).

Example 2

Generation of *Dutt1/Robo1*^{-/-} mice (see Fig. 1). A genomic library of E14 TG2a ES 15 cell DNA cloned in lambda2001 and was screened with a mouse *Dutt1/Robo1* cDNA clone corresponding to exon 2 and positive clones purified and subcloned. An 8.0 kb *SacI* fragment containing exon 2 of the mouse *Dutt1/Robo1* gene was used for construction of the targeting vector. A neo expression cassette (16) (pMC1-neo poly[A]), corrected to the wild-type neo sequence (17), was used to replace a 0.7 kb 20 genomic *HindIII*, *BamHI* fragment spanning sequences coding for exon 2 of the *Dutt1* gene. The replacement sequence was flanked 5' by 4.4 kb and 3' by 1.4 kb of genomic sequence. A thymidine kinase gene expression cassette (18) was ligated to a unique *XbaI* site at the 5' of the longer homologous arm. The final vector was linearized with *HindIII* for electroporation. CCB ES cells were maintained on mouse 25 embryonic feeders. Twenty-five micrograms of *HindIII*-digested targeting vector DNA were used to electroporate 1×10^7 CCB ES cells. *SacI* digested genomic DNA from cell clones surviving G418 (GIBCO) at 400 μ g/ml and Gancyclovir at 2.5 μ M selection was subjected to Southern blot analysis. Positively targeted clones were confirmed using a 5' 0.7 kb *BamHI* fragment internal probe and a 0.4 kb 30 *HindIII/SacI* probe external to the homologous sequence on genomic DNA digested with *SacI*. ES cells from two independent clones were used for injection into blastocysts derived from C57BL/6 mice. Blastocysts were transferred to pseudo-

pregnant females, and chimaeric offspring were detected by the presence of agouti colour on a non-agouti background. Chimaeric males were mated to C57BL/6 females to produce ES cell-derived offspring. Their genotype was confirmed by Southern blot analysis of tail DNA. Mice heterozygous for the gene targeting event, i.e. with a deletion of exon 2 of the *Dutt1/Robo1* gene, were inter-crossed to generate homozygotes.

Example 3]

RNA analysis. Total RNA was prepared from various tissues with Trizol Reagent (GIBCO BRL). Reverse transcription reactions were performed on total RNA (5 micrograms) in 20 microlitres containing 0.5 micrograms of random hexamer (Pharmacia) 25 mM Tris-HCl, pH 8.3, 50 mM KCl, 2.0 mM dithiothreitol, 5.0 mM MgCl₂, 1 mM each of dATP, dCTP, dGTP & dTTP, 1 u/microlitre of RNasin ribonuclease inhibitor (Promega, Madison, WI), and 10 u/microgm of SUPER RT (HT Biotechnology Ltd, Cambridge, England). Each RT reaction was at 42°C for 40 minutes. The product of RT was diluted five-fold and amplified by PCR. (30 cycles of 1min denaturation at 95°, 1min annealing at 55° and 1min extension at 72°). The primers used were: exon 1 forward 5'-AGGGATTGACAAGCCTCCGG-3', exon 2 reverse 5'-AGCTACCTCCAGCGATGCGT-3', exon 3 reverse 5'-CATCTTTATCATCCAGGGGT-3'. The products were visualised following electrophoresis on agarose gels.

Example 4

Western blotting. Protein lysates were prepared with a lysis buffer composed of 9 M urea, 75 mM Tris-HCl pH 7.5 and 0.15 M beta-mercaptoethanol as described (19). Protein concentration was determined using the Bradford assay, (Bio-Rad Laboratories). For Western blotting, 50 microgm of protein lysate were subjected to SDS-PAGE on a 10% polyacrylamide gel and transferred onto a nitrocellulose membrane (BioTrace; Gelman Sciences). Transfer was assessed by Ponceau S staining (Bio-Rad). Filters were incubated with a polyclonal antisera raised against the C-terminal peptide of DUTT1/ROBO1 (CYERGEDNNEELEETES) followed by

HRP-conjugated mouse anti-rabbit IgG and visualised by enhanced chemiluminescence (Amersham, Pharmacia).

Example 5

5 **Histological analysis and immunohistochemistry.** Tissues or whole embryos were fixed in 4% paraformaldehyde in PBS buffer for at least 24h, paraffin embedded and processed to give 5 micrometre sections. Sections were stained with haematoxylin and eosin (H&E). For immunohistochemistry, *Dutt1/Robo1* was detected using the polyclonal antisera described above followed by donkey biotin-conjugated anti-
10 rabbit secondary antibody and Cy-3 conjugated Streptavidin. Slides were mounted with Vectashield-mounting medium. Surfactant protein-C was detected using a commercially available antibody (SantaCruz) followed by donkey biotin-conjugated anti-goat secondary antibody and Cy-3 conjugated Streptavidin.

15 **Example 6**

Generation of *Dutt1/Robo1* mutant mice. The *Dutt1/Robo1* gene was disrupted in mice by targeted replacement of exon 2 with the neomycin phosphotransferase gene, producing a mutant form of the gene unable to code for the first immunoglobulin domain (Fig.1A). This exactly reproduces a truncated *DUTT1/ROBO1* transcript
20 detected in the human lung cancer cell line NIH-H219X (9). Following transfection, DNA from G418-resistant embryonic stem cell (ES) clones was analysed by Southern blotting to identify correctly targeted clones by hybridisation with probe (a) within the targeting construct (Fig. 1B) and with probe (b) external to the homologous integration. Two such clones were injected into blastocysts and
25 resulting chimaeras bred with C57BL/6 mice. Tail DNA from offspring was screened by Southern blotting to identify mice carrying the mutant form of the gene in their germ-line (Fig.1C). Heterozygotes were interbred to produce homozygous offspring. RT-PCR was used to confirm that exon 1 and 3 sequences were contiguous and that exon 2 was absent in the transcript from *Dutt1/Robo1*^{-/-} mice
30 (result not shown).

Example 7

Dutt1/Robo1 protein in mutant and normal mice. In all organs from homozygous offspring examined by Western blotting (embryos and new-born mice), the resulting protein was reduced to about one half of the level detected in wild-type organs (Fig. 2A). The Dutt1/Robo1 protein is shorter by 20kDa due to loss of the first immunoglobulin domain encoded by exon 2 (Fig. 2A). Immunohistochemistry was performed using a polyclonal antibody raised against the C terminus of human DUTT1/ROBO1 (Clark, KJ, JX, EH and PHR unpublished observations), which recognises the mouse protein. This detected the protein at high levels in epithelial cells lining bronchi but at low or undetectable levels in adjacent mesenchyme and cells lining alveoli (Fig. 2B).

Example 8

Dutt1/Robo1^{-/-} mice frequently die at birth due to respiratory failure. Mice heterozygous for the *Dutt1/Robo1* deletion were born at the expected frequency and had no obviously abnormal phenotype. At birth, *Dutt1/Robo1^{-/-}* mice failed to feed (Fig 3), were usually inactive with laboured breathing and nearly two-thirds (22/36) died within the first twenty-four hours. At autopsy, lungs were frequently dark red (Fig. 3) and sank in fixative suggesting inadequate inflation. Following fixation and H&E staining, lungs from *Dutt1/Robo1^{-/-}* mice and their normal littermates were compared. The most striking feature of lungs from *Dutt1/Robo1^{-/-}* newborn mice was increased cellularity of the mesenchyme resulting in reduction of the size of terminal air spaces (Fig. 4D); alveolar septa were reduced in number and thicker than in the wild-type littermates (Fig 4C); the bronchioles were of an irregular cross section (Fig. 4D). The overall appearance was consistent with a developmental delay of 0.5 to 1.0 day. To determine if the abnormal phenotypes were present before birth, lungs from day E15.5 and day 18.5 were similarly examined. This showed that the abnormal lung morphology was already established at day 15.5 (Fig. 4A&B).

Example 9

Search for other phenotypic changes in *Dutt1/Robo1^{-/-}* mice. All newborn mice which die at birth were examined for macroscopic abnormalities in addition to their abnormal lungs. None was found except for three mice with diaphragmatic hernias.

The heart, kidneys and muscle were examined microscopically but no abnormal histology was detected. In view of the function of *Drosophila Robo* in the control of axonal migration and the high level of expression of the protein in the brain of newborn mice (Fig. 2A) and in foetal brain (Clark, KJ, JX, EH and PHR unpublished observations), brains and spinal chords of mutant mice were examined. Transverse serial sections of spinal chord from E12.5 and E15.5 embryos were examined by H&E staining for thickening of the ventral commissures underlying the floor plate of the spinal chord. However, no differences could be detected between *Dutt1/Robo1^{-/-}* and normal embryos (results not shown).

Example 10

Development of bronchial epithelial hyperplasia in *Dutt1/Robo1^{-/-}* mice surviving birth.

Our results show that the *Dutt1/Robo1* targeted germline mutation, which emulated the naturally occurring somatic mutation found in the NCIH219X lung tumour cell line, has a profound effect on lung development. Some homozygous mice do survive to adulthood and are capable of breeding (39%). The *Dutt1/Robo1^{-/-}* mice surviving beyond the first 24h after birth appeared to develop normally but commonly showed signs of morbidity at ages ranging from three weeks onwards. Following autopsy and tissue examination, bronchial epithelial hyperplasia was observed as a common feature. In some lungs, papillary hyperplasia was seen throughout the entire bronchial tree (Fig. 4F&H), contrasting with the uniform cuboidal appearance of the bronchial epithelium of normal adult mice (Fig. 4E&G). Very occasionally, focal dysplasia was observed characterised by increased epithelial cell layers, large pleomorphic nuclei and reduced cytoplasm (Fig. 4I). Thus while differences in gross lung morphology are less distinct in surviving post-natal homozygotes, specific bronchial epithelial alterations are apparent in these mice.

Example 11

Production and purification of soluble Fabs

Soluble Fab's were produced as described by Roovers *et al* (1998). The cultures were inoculated with *E. coli* TG1 (K12, D(*lac-pro*), *supE*, *thi*, *hsdD5/F'* *traD36*, *proA⁺B⁺*, *lacI^f*, *lacZDM15*) harbouring the Fab in pCES1. Individual bacterial clones were

picked and production of soluble Fab was induced by activation of the upstream *Lac Z* promoter with isopropyl- β -1-thiogalactopyranoside (IPTG) as described by Marks *et al* (1991).

5 ELISA

An ELISA using soluble Fabs was performed on purified, recombinant DUTT1 in order to identify binding Fabs from the individual clones selected. ELISA plates (Costar, Cambridge, MA, USA) were coated overnight with 1 $\mu\text{g ml}^{-1}$ DUTT1 in phosphate-buffered saline (PBS), washed three times with PBS-T [PBS, 0.5% (v/v) Tween 20], three times with PBS and blocked for 1 h at room temperature (RT) with 2% MPBS [2% (w/v) Marvel - skimmed milk powder - in PBS]. After blocking, induced bacterial supernatants were added [50% (v/v) in 2% MBS] and incubated for 1.5 h at RT. Bound antibody fragments were detected with a further antibody [50% (v/v) hybridoma supernatant in 2% MPBS], peroxidase-conjugated rabbit anti-mouse immunoglobins [Dako, Glustrup, Denmark; 0.1% (v/v) in 2% MPBS] and stained with trimethylbenzidine (TMB) and hydrogen peroxide. Optical density was measured at 450 nm. This assay is described in detail by Roovers *et al* (1998 *ibid*).

Competition ELISA

20 For competition ELISA, DUTT1 binding of soluble Fab antibody fragments was detected in the presence of excess whole murine monoclonal antibody.

Specificity ELISA

The assay was carried out as described above but the wells were coated with antigens such as 10 $\mu\text{g/ml}$ Bovine Serum Albumin (Sigma) in PBS, 3 mg/ml Hen Egg White Lysozyme (Boehringer Mannheim) in 0.1 M NaHCO_3 (pH 9.6), 10 $\mu\text{g/ml}$ Tetanus Toxoid in 0.1 M NaHCO_3 (pH 9.6) and 0.5 $\mu\text{g/ml}$ EGP-2 in PBS.

Example 12**Polyclonal antibody and Immunohistochemistry**

A polyclonal antisera was raised in rabbits against the C-terminal peptide of DUTT1/ROBO1 (CYERGEDNNEELEETES) by Zeneca (Cambridge Research
5 Biochemicals UK). The antisera specifically recognised a band at approximately 190kDa in cells transfected with either a DUTT1 or ROBO1 expression vector. Staining was blocked by preincubation of the immunising peptide with the antisera.

Tissues or whole embryos were fixed in 4% paraformaldehyde in PBS buffer for at
10 least 24h, paraffin embedded and processed to give 4 micrometre sections. Sections were deparaffinised and endogenous peroxide quenched. After blocking, Dutt1/Robo1 was detected using the polyclonal antisera described above followed by donkey biotin-conjugated anti-rabbit secondary antibody (SantaCruz USA) and amplification with the Vectastain ABC kit (Vector Laboratories USA) as per the manufacturers protocol.
15 Positive staining was detected using nickel enhanced diaminobenzidine tetrahydrochloride (DAB) and counterstained with Fast Red. Slides were mounted with Vectashield-mounting medium. Staining was blocked by preincubation of the immunising peptide with the antisera. Sections were stained with haematoxylin and eosin (H&E).

20 All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in
25 connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

CLAIMS

1. A method for the early detection of cancer in a population of cells comprising the
5 steps of:
 - (1)providing a population of cells
 - (2)assaying the cell population for an increased level of DUTT1 in any one or more of those cells as compared with normally dividing cells.
- 10 2. A method for the early detection of cancer in a population of cells comprising the steps of :
 - (1)providing a population of cells
 - (2)assaying those cells and establishing a reference level of DUTT1;
 - (3)obtaining a population of cells for diagnosis,
 - 15 (4)assaying the cell population of step 3, for an increased level of DUTT1 in any one or more of those cells when compared with the reference level of DUTT1.
- 20 3. A method for the diagnosis of cancer in a population of cells comprising the steps of:
 - (1)providing a population of cells
 - (2)assaying the cell population for an increased level of DUTT1 in any one or more of those cells as compared with normally dividing cells.
- 25 4. A method for the diagnosis of cancer in a population of cells comprising the steps of :
 - (1)providing a population of cells
 - (2)assaying those cells and establishing a reference level of DUTT1,
 - (3)obtaining a population of cells for diagnosis,
 - 30 (4) assaying the cell population of step 3, for an increased level of DUTT1 in any one or more of those cells when compared with the reference level of DUTT1.

5. A method according to any preceding claim which is an in vitro method.
6. A method according to any preceding claim wherein the cancer is lung cancer.
- 5 7. A method according to claim 6, wherein the cancer is human lung cancer.
8. A method according to any preceding claim wherein the cells for diagnosis include bronchial epithelial cells and/or bronchial hyperplasia of epithelium
- 10 9. The use of a DUTT1 binding agent in the diagnosis of cancer.
10. The use according to claim 9, which has any of the features of claims 5 to 8.
- 15 11. A method for the selective labelling of cancer cells comprising the step of treating one or more cancer cells with a DUTT1 binding agent.
12. A method according to claim 11 wherein the cancer cells are lung cancer cells
- 20 13. A method according to claim 11 or claim 12 wherein the cells are human lung cancer cells.
14. A method according to any of claims 11 to 13 wherein the cells are primary cancer cells.
- 25 15. A method according to any one of claim 11 to 13 wherein the cancer cells are metastatic cells.
16. A method according to any of claims 11 to 15 wherein the DUTT1 binding agent is any one or more selected from the group consisting of: anti-DUTT1 antibodies, DUTT1 binding peptides and small synthetic molecules which bind to DUTT1.
- 30

17. A method according to claim 16 wherein the DUTT1 binding agent further comprises or has associated with it detection means.
18. A method according to claim 17 wherein the detection means allows detection of cancer cells within an in vivo environment.
19. A method according to claim 18 wherein the in vivo environment is the human body.
20. The use of a DUTT1 binding agent in the selective labelling of cancer cells.
21. The use according to claim 20, wherein the DUTT1 binding agent is any one or more selected from the group consisting of: anti-DUTT1 antibodies, DUTT1 binding peptides and small synthetic molecules which bind to DUTT1.
22. The use according to claim 20, wherein the DUTT1 binding agent has any of the features of claims 16 to 18.
23. The use according to claim 21 to claim 22, in the in vivo imaging of cancer cells.
24. A method for the imaging of cancer cells comprising the step of treating one or more cancer cells with one or more DUTT1 binding agent/s wherein the DUTT1 binding agent further comprises detection means.
25. A method according to claim 24, for the in vivo imaging of cancer cells.
26. A method according to claim 24 or 25 for the imaging of cancer cells selected from the group consisting of: human lung cancer cells, human epithelial cancer cells, human sarcoma cells, human haematopoietic cancer cells
27. A composition comprising DUTT1, and/or a binding agent thereof.

28. Use of DUTT1, and/or a binding agent thereof in the preparation of a medicament for the prophylaxis or treatment of cancer.

5 29. Use according to claim 28, of a DUTT1 binding agent.

30. Use according to claim 28 where the DUTT1 binding agent is an antibody raised against DUTT1.

10 31. Use according to any of claims 27 to 29 in the preparation of a medicament for the treatment of lung cancer.

32. Use according to claim 31 in the preparation of a medicament for the treatment of human lung cancer.

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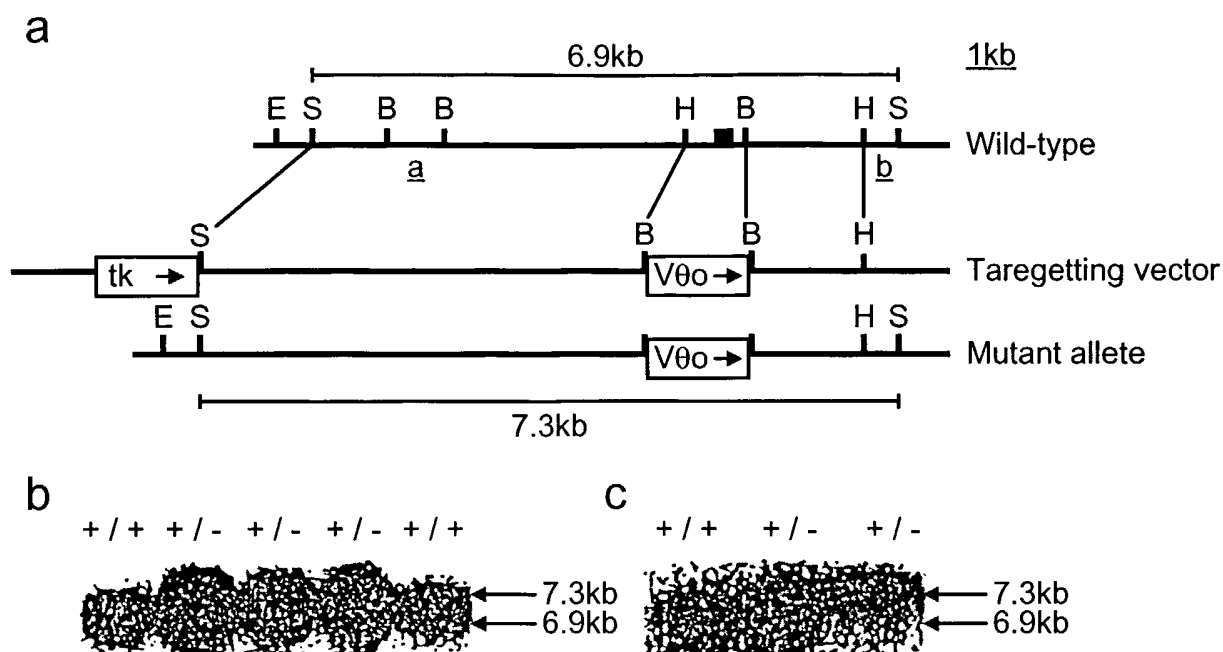


FIG. 1

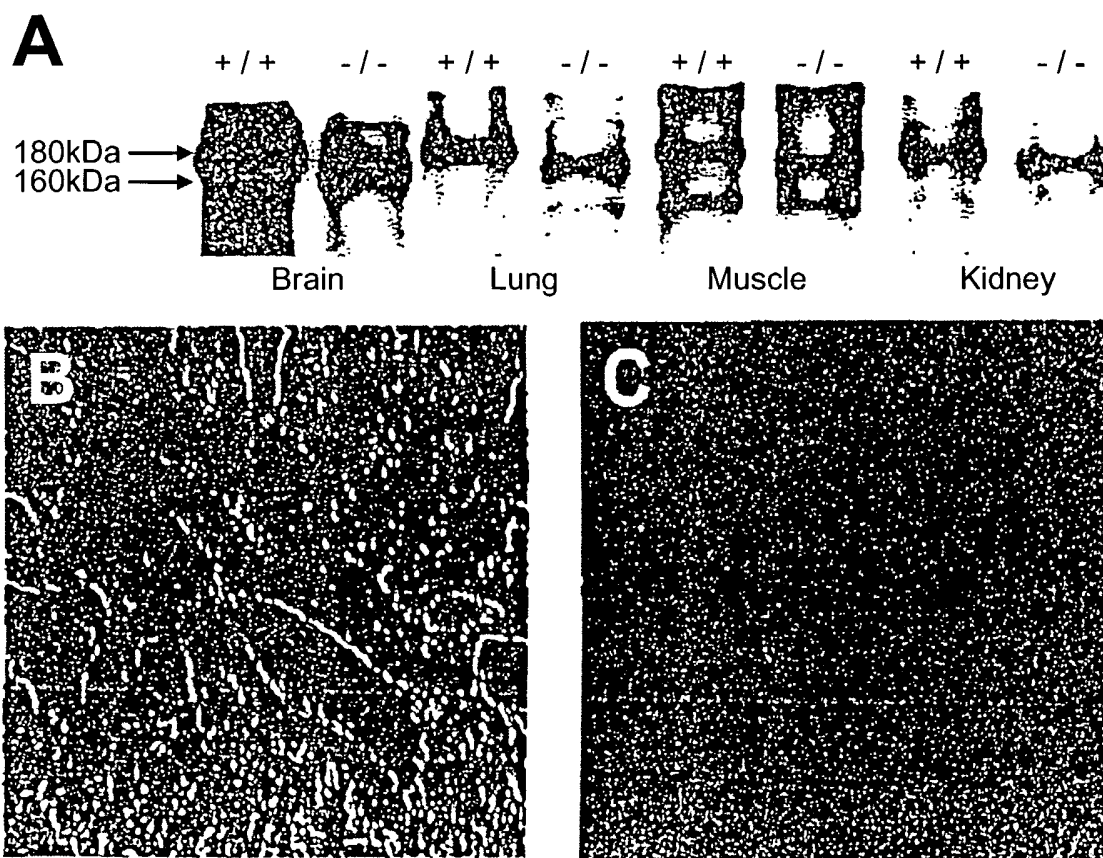


FIG. 2

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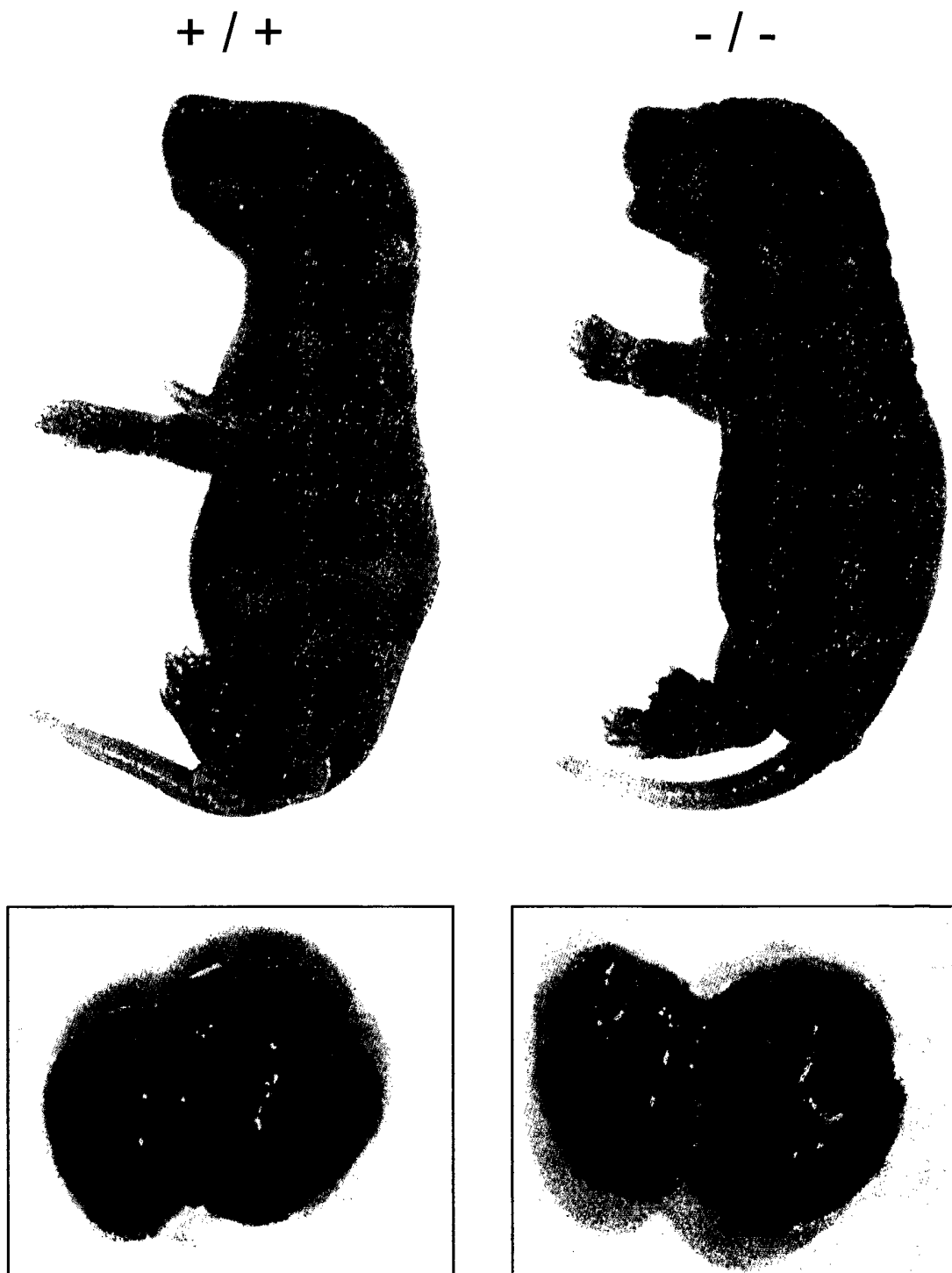


FIG. 3

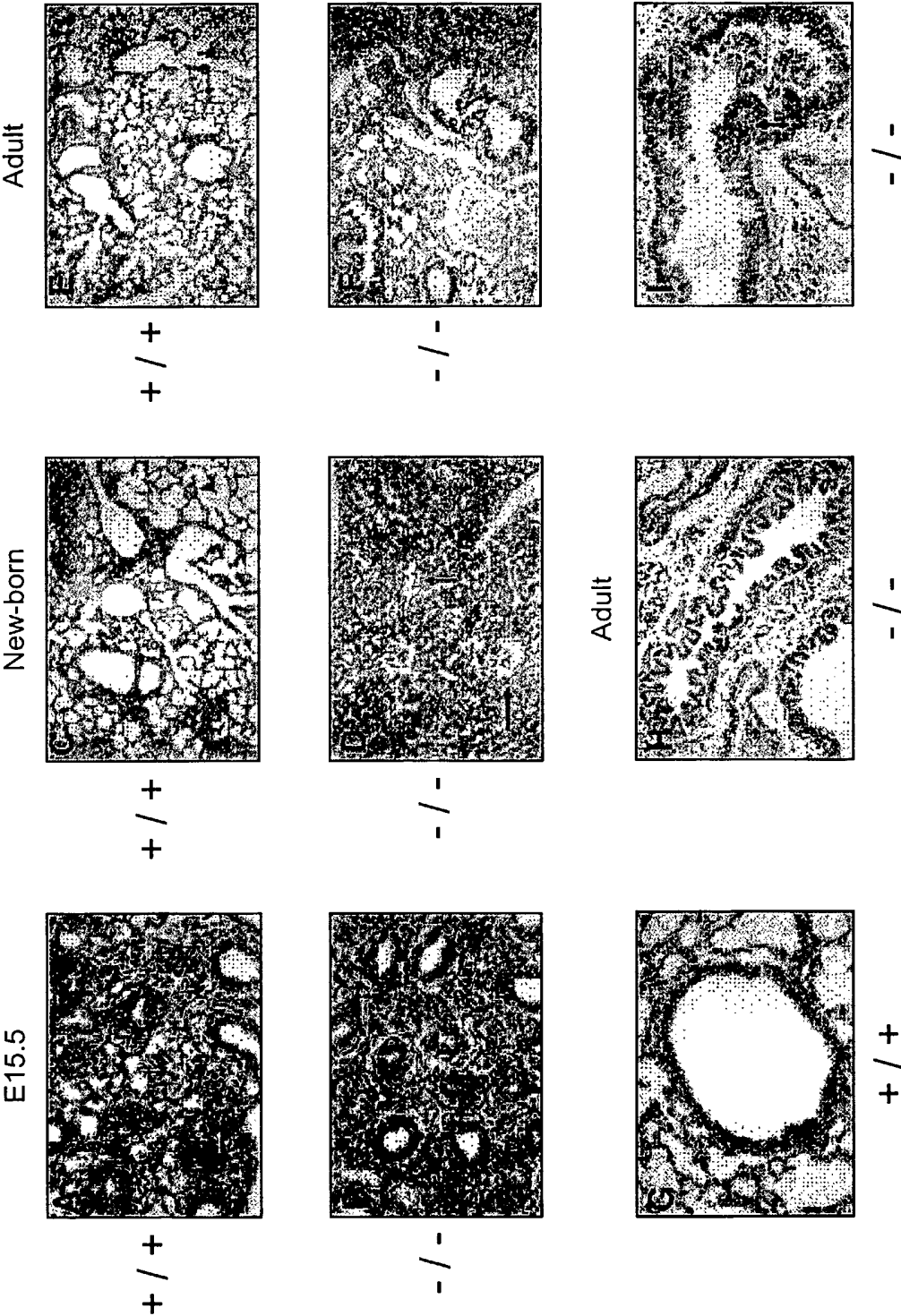


FIG. 4

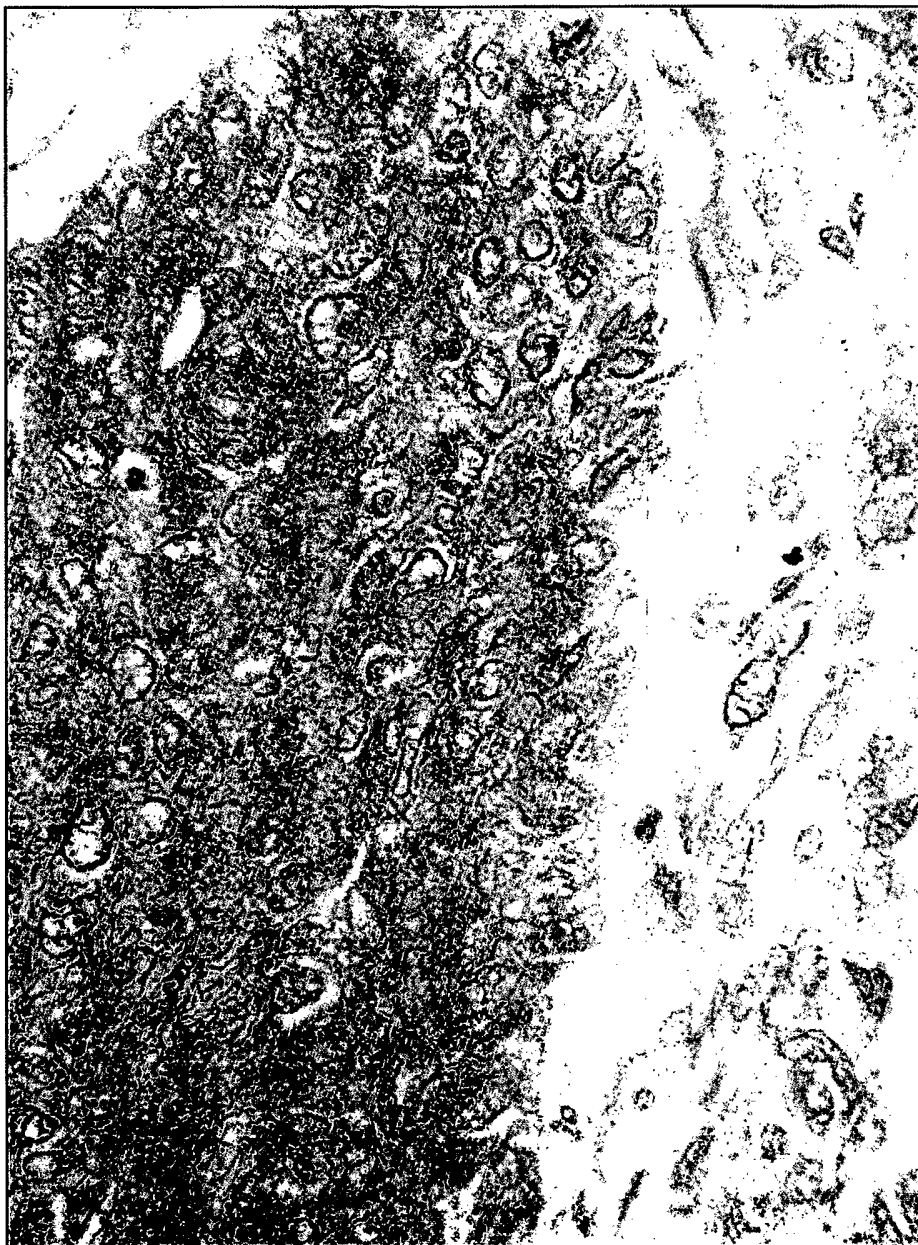


FIG. 5

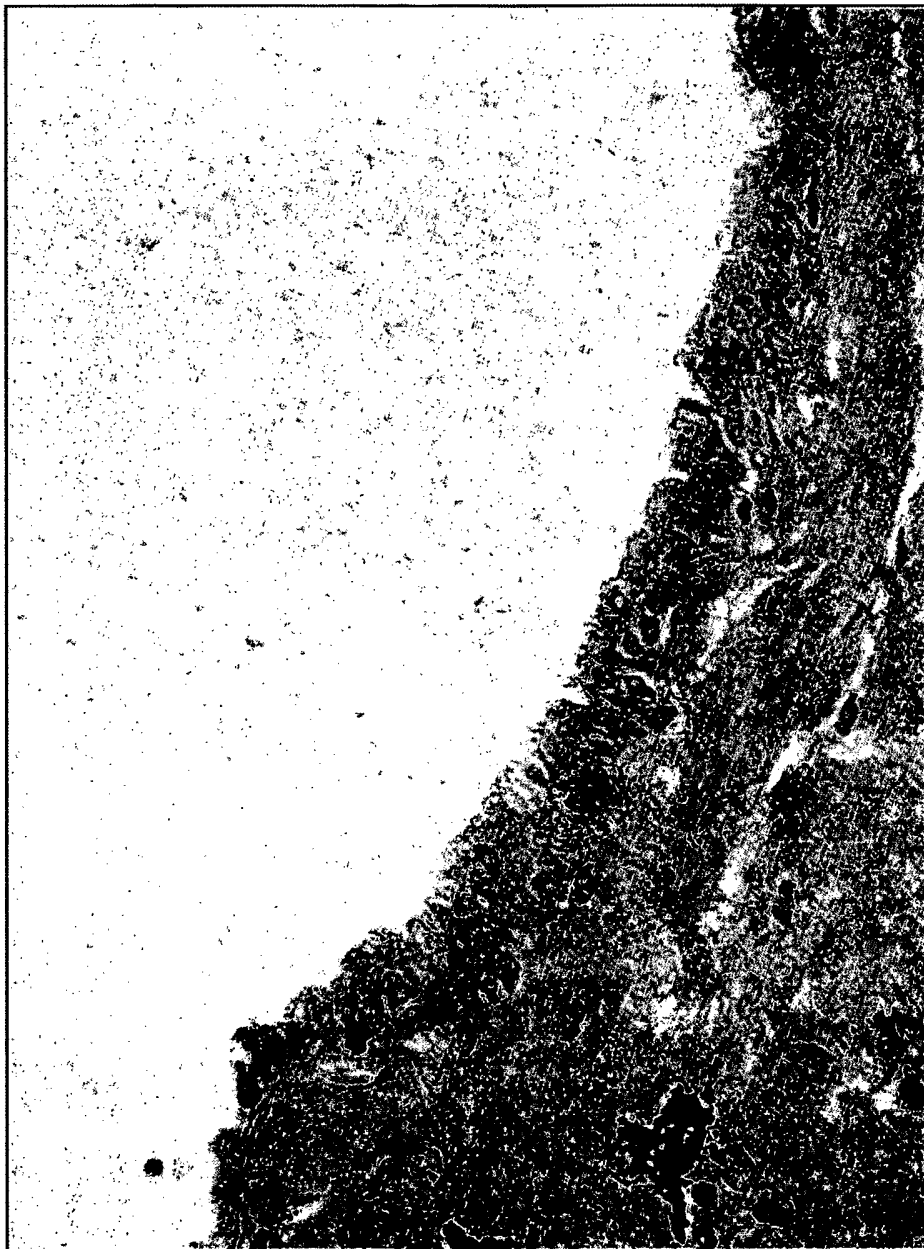


FIG. 6