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[CA/CA]; University-Industry Liaison Office, #103 - 6190
Agronomy Road, Vancouver, British Columbia V6T 1Z3
(CA).

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(71) Applicant (for all designated States except US): **THE
UNIVERSITY OF BRITISH COLUMBIA** [CA/CA];
University-Industry Liaison Office, #103 - 6190 Agron-
omy Road, Vancouver, British Columbia V6T 1Z3 (CA).

(72) Inventor; and

(75) Inventor/Applicant (for US only): **DUNN, Sandra E.**

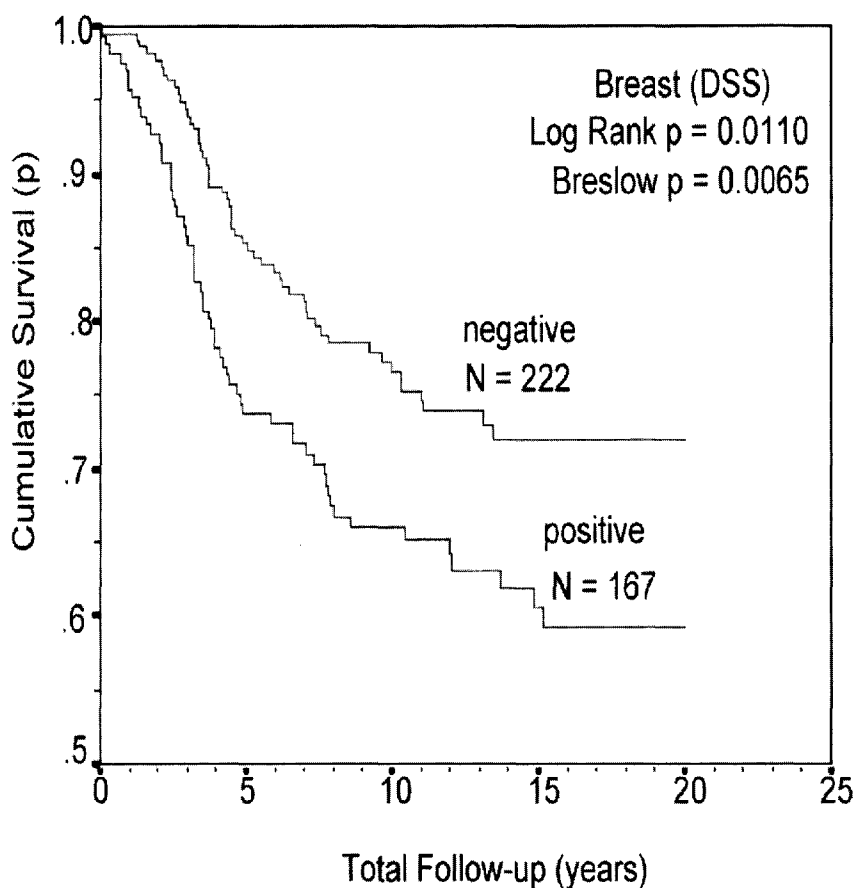
(74) Agent: **SMART & BIGGAR**; Suite 2200, 650 West
Georgia Street, Box 11560, Vancouver Centre, Vancouver,
British Columbia V6B 4N8 (CA).

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(54) Title: THERAPEUTIC YB-1 PHOSPHORYLATION DECOYS



(57) Abstract: In various aspects, the invention provides cell permeable Y-box binding protein-1 (YB-1) phosphorylation inhibitors, such as compounds comprising a peptidic sequence that is YB-1 selective protein kinase B (Akt) phosphorylation site. Compounds of the invention may take the form of peptide therapeutics for use, for example, in treating cancers.

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THERAPEUTIC YB-1 PHOSPHORYLATION DECOYS

FIELD

[0001] Aspects of the invention relate to inhibitors of transcription factor phosphorylation, more specifically, selective peptidic inhibitors of YB-1 phosphorylation that have therapeutic activity.

BACKGROUND

[0002] Y-box binding protein-1 (YB-1) is a transcription and translation factor that alters the expression of at least ten genes associated with drug resistance and tumour cell growth (JANZ *et al* 2002, *Int J Cancer* 97:278-282; JURCHOTT *et al* 2003, *J Biol Chem* 278:27988-27996; EN-NIA *et al* 2004, *J Biol Chem* 280:7702-7711; SHIBAO *et al* 1999, *In J Cancer* 83:732-737). The YB-1 protein is highly expressed in cancers of the breast (BARGOU *et al* 1997, *Nature Med* 3: 447-450), prostate (GIMENEZ-BONAFE *et al* 2004, *Prostate* 59:337-349, colon (SHIBAO, *supra*), ovary (YAHATA *et al* 2002 *J Cancer Res Clin Oncol* 128:621-626) and bone (ODA *et al* 1998 *Clin Cancer Res* 4: 2273-2277). In some breast cancers, expression of YB-1 is associated with particularly aggressive tumors and a prognosis of poor survival (RUBENSTEIN *et al* 2002 *Cancer Res.* 62:4985-4991; JANZ, *supra*). YB-1 is not expressed at detectable levels in normal tissues (BARGOU, *supra*; RUBENSTEIN, *supra*).

[0003] YB-1 was originally isolated in an attempt to identify DNA binding proteins that interact with the epidermal growth factors EGFR and HER2 (SAKURA *et al* 1988, *Gene* 73:499-507). At the cellular level, YB-1 resides primarily in the cytoplasm but translocates to the nucleus, in a yet-undefined manner, where it binds to promoters with a 5'- CTGATTG -3' motif. In the cytoplasm, YB-1 interacts with RNA to regulate translation (EVDOKIMOVA *et al* 2001 *EMBO J* 20:5491-5502), while in the nucleus it binds to DNA and controls transcription (KUWANO *et al* 2003, *Cancer Sci* 94:9-14). As a transcription factor, YB-1 has been characterized for its ability to regulate at least ten genes that promote tumour growth and/or drug resistance. Among

the most notable genes are *cyclin A*, *cyclin B*, *egfr* and *her-2*. YB-1 also partners with other transcription factors such as p53 (*LASHAM et al 2003 J Biol Chem 355:16-35523*) and AP-2 (*MERTENS et al 1998 J Biol Chem 273:32957-32965*), and with cofactors (e.g., p300), to regulate gene expression (*HIGASHI et al 2003 J Biol Chem 278:43470-43479*).

[0004] YB-1 is phosphorylated at serine 102 by AKT, and this phosphorylation event is important in nuclear trafficking and DNA binding of YB-1. Inhibition of this phosphorylation halts tumor cell growth (*SUTHERLAND et al 2005 Oncogene 24:4281-4292*). The effect of YB-1 on cell growth may be cell-type specific, as YB-1 has demonstrated growth-suppressing effects in chicken embryo fibroblasts (*BADER et al 2003. Proc Nat Acad Sci 100:12384-12389*). YB-1's expression across several cancer types is consistent with this. Tumor cells are dependent on YB-1 - melanoma, adenocarcinoma, hepatoma, fibrosarcoma, colon cancer and breast tumor cells die when its expression is knocked out with antisense RNA (*LASHAM supra*).

[0005] The human epidermal growth factor receptors (HER1/EGFR, HER2, HER3 and HER4) are important for sustaining the growth of breast cancer cells (*YARDEN 2001. Oncology 61:1-13*). These receptors form homodimers and heterodimers to activate signal transduction in response ligands such as EGF, amphiregulin, and heregulin. EGFR and HER2 are over-expressed in a subset of breast cancers (*NIELSEN et al. 2004 Clin Cancer Res 10:5367-5374; SLAMON et al 1987, Science 235:177-182*). Over-expression of EGFR is generally due to transcriptional activation (*KERSTING et al 2004. Lab Invest 84:582-587*), and a number of *cis*- and *trans*- activating elements regulating EGFR expression have been identified, including the EGF-responsive DNA binding protein-1, c-JUN, and SP-1 (*CHEN et al 1993. Cell Growth Diff 4:975-983; ZENZ et al 2003. Dev Cell 4:879-889; KAGEYAMA et al 1988 J Biol chem. 263:6329-6336*). YB-1 has also been shown to regulate

EGFR in human mammary epithelial cells (*BERQUIN et al 2005, Oncogene 25:3177-3186*).

[0006] The widespread involvement of YB-1 in multiple tumour types suggests a central role in the regulation of growth and development of cancers.

[0007] YB-1 has been implicated as having a causative or pathological role in a variety of diseases (which may therefore be identified as YB-1 mediated diseases), apart from cancer, including: heart disease (*Kamalov et al 2005, Biochem Biophys Res Commun. 19;334(1):239-44*; *Zhang et al 2005, Mol Biol Cell. 16(10):4931-40*); kidney disease (*van Roeyen et al 2005, J Am Soc Nephrol. 16(10):2985-96*); diabetes (*Fukada and Tonks 2003, EMBO J. 3;22(3):479-93*); autoimmune disease (*Seko et al 2006, Autoimmun Rev. 5(5):299-305*); cerebral palsy (*Liverman et al 2006, Neurosci Lett 22;399(3):220-5*); and, infection (including viral infection) (*Chen et al 1995, Proc Natl Acad Sci U S A. 14;92(4):1087-91*).

[0008] Protein transduction domains (PTDs) have been used to deliver a wide variety of therapeutics, such as protein-based drugs for treating breast cancer (*Harada et al 2006, Breast Cancer 13(1):16-26*). Similarly, liposomal formulations, including liposomal formulations that are decorated with protein transduction domains, may be useful for delivering protein-based drugs (*Torchilin et al 2001, Proc Natl Acad Sci USA 98:8786-8791*). An extensive reference list is provided below, incorporated herein by reference, documenting the use of protein transduction domains and related molecules. Table 1 sets out a number of protein transduction domains described in the literature.

Table 1

CPP	Origin	sequence	reference
Tat (48-62)	HIV-1 transactivator of	GRKKRRQRRRPPQ	Vives 1997

	transcription (48-62)		
Tat (49-59)	HIV-1 transactivator of transcription (49-59)	YGRKKRRQRRR	Nagahara 1998
Antp, penetratin	Antennapedia homeodomain (43-58)	RQIKIWFQNRRMKWKK	Derossi 1994
VP22	Herpes simplex virus	NAATATRGRSAASRP TQRPRAPARSAS RPRRPVQ	Elliott 1997
galparan, transportan	galanin + mastoparan	GWTLNSAGYLLGKINL KALAALAKKIL	Pooga 1998
MPG	gp41 fusion sequence – SV40 NLS	GALFLGFLGAAGSTM GAWSQPKSKRKV	Morris 1997
	<i>Caiman crocodylus</i> Ig(v) light chain – SV40 NLS	MGLGLHLLVLAAALQG AWSQPKKKRKV	Chaloin 1998
SynB1	protegrin	RGGRLSYSRRRFSTS TGR	Rousselle 2000
Fushi-tarazu	Fushi-tarazu (254-313)	SKRTRQTYTRYQTLEL EKEFHFNRYITR RRRIDIANALSLSERQI KIWFQNRRMKSK KDR	Han 2000
Engrailed	Engrailed (454-513)	EKRPRTAFSSEQLARL KREFNENRYLTE RRRQQLSSELGLNEA QIKIWFQNKRAKI KKST	Han 2000
pVEC	murine vascular endothelial cadherin (615-632)	TLLILRRRIRKQAHAAH S	Elmqvist 2001
plsl	Plsl (183-243)	RVRTLNEKQLHTLRT CYAANPRPDAL	Kilk 2001

		MKEQLVEMTGLSPRVI RVWFQNKRCCKD KKRSIMM	
KALA	Model amphipathic peptide	WEAKLAKALAKALAKH LAKALAKALKAC EA	Wyman 1997
KLAL	Model amphipathic peptide	KLALKLALKALKKAALKL A	Oehlke 1998

SUMMARY OF THE INVENTION

[0009] In various aspects, the invention provides analogues of the YB-1 Akt phosphorylation site that are capable of inhibiting pathological cellular activities. In selected embodiments, the invention provides cell permeable YB-1 phosphorylation inhibitors having a peptidic sequence that is a YB-1 selective Akt phosphorylation site.

[0010] In accordance with one aspect of the invention, there is provided a method of inhibiting YB-1 mediated transcription, the method comprising contacting a cell with an inhibitor of Akt mediated YB-1 phosphorylation, so that transcriptional activation of YB-1 is in turn inhibited.

[0011] In accordance with another aspect of the invention, there is provided a method of treating a YB-1 expressing cell, such as a cancer cell, for example a cell in a patient or test subject, the method comprising administering a chemotherapeutic regimen comprising an inhibitor of YB-1 phosphorylation or YB-1 mediated transcription.

[0012] In accordance with another aspect of the invention, there is provided a method of modulating metastasis of a cell expressing YB-1, the method comprising contacting a cell with an inhibitor of YB-1 phosphorylation and transcriptional activation.

[0013] In some embodiments, the inhibitor of YB-1 phosphorylation or YB-1 mediated transcription may be a fragment of YB-1, or may be a peptide substantially similar to a fragment of YB-1. In other embodiments, the inhibitor of YB-1 phosphorylation or transcriptional activation may be a peptidomimetic

[0014] In accordance with another aspect of the invention, the inhibitor of YB-1 phosphorylation may be a peptide according to SEQ ID NO: 12, or a peptide substantially similar to SEQ ID NO: 12.

[0015] In accordance with another aspect of the invention, the inhibitor of YB-1 phosphorylation or YB-1 mediated transcription may be an antibody that binds a protein having a sequence according to SEQ ID NO: 12, or a sequence substantially similar to SEQ ID NO: 12.

[0016] In selected embodiments, a YB-1 expressing cell, such as a cancer cell, may be treated with compounds of the invention. Cancers or cancer cells amenable for treatment may for example be identifiable as a cancer of the pancreas, breast, thyroid, ovary, uterus, testis, prostate, thyroid, pituitary gland, adrenal gland, kidney, stomach, esophagus or rectum, head and neck, bone, nervous system, skin, blood, nasopharyngeal tissue, lung, urinary tract, cervix, vagina, exocrine glands or endocrine glands, or a cancer of unknown primary site.

[0017] In accordance with another aspect of the invention, there is provided a method of screening for an inhibitor of YB-1 phosphorylation or transcriptional activation, the method comprising: providing a system comprising a reporter gene construct having a reporter gene and a YB-1 transcriptional activation sequence and transcriptionally active YB-1; providing an inhibitor of YB-1 transcriptional activation; providing a test compound; and

contacting said system with said test compound and determining whether said test compound modulates YB-1 transcriptional activation.

5 **[0018]** In accordance with another aspect of the invention, there is provided a polypeptide comprising an amino acid sequence substantially similar to the sequence SEQ ID NO: 12.

10 **[0019]** In accordance with another aspect of the invention, there is provided a use of a polypeptide comprising an amino acid sequence substantially similar to the sequence SEQ ID NO: 12 for the preparation of a medicament, for example for use in a chemotherapeutic regimen.

15 **[0020]** In accordance with another aspect of the invention, there is provided a use of a polypeptide comprising an amino acid sequence substantially similar to the sequence SEQ ID NO: 12 for the preparation of a medicament for use in a radiotherapeutic regimen.

20 **[0021]** In accordance with another aspect of the invention, there is provided a use of a polypeptide comprising an amino acid sequence substantially similar to the sequence SEQ ID NO: 12 for the preparation of a medicament for use in a combination chemotherapeutic regimen.

25 **[0022]** In accordance with another aspect of the invention, there is provided a use of a polypeptide comprising an amino acid sequence substantially similar to the sequence SEQ ID NO: 12 for the preparation of a medicament for use in an alternative therapeutic regimen.

BRIEF DESCRIPTION OF THE DRAWINGS

30 **[0023]** **Figure 1: The impact of YB-1 expression on patient survival.** A total of 389 tumours were examined for YB-1 expression and patient survival was monitored over 20 years. Patients with tumours expressing YB-1 were less likely to survive long term based on log rank and Breslow (Gehan-

Wilcoxon) survival analyses. Survival was based on disease specific survival (DSS).

[0024] Figure 2: YB-1 binds directly to the EGFR promoter. A)

5 Schematic representation of potential YB-1 EGFR binding sites and the location of the primers used for ChIP. **B)** DNA:protein complexes were cross-linked from MCF-7/Flag:YB-1 and MCF-7/Flag:YB-1(Ala102) cells. ChIP of Flag:YB-1 and Flag:YB-1(Ala102) from MCF-7 cells using an anti-Flag M2 antibody. The immunoprecipitated DNA from Flag:YB-1 (lanes 1-4) or YB-1(Ala102) (lanes 5-8) were amplified using sequence specific primers EGFR 1b, 2a, 2b and 3. Flag:YB-1 bound to several of the loci on the EGFR promoter while loss of the Ser102 site by mutation to Ala102 disrupted binding to the EGFR1b and EGFR2a sites. The IgG negative control was also evaluated using each of the EGFR primer sets (lanes 9-12). Input DNA for each sample was also titrated (1, 3, 5 ul) and amplified with EGFR 2a primers to demonstrate that the sample input was equivalent (Lanes 13-18). An aliquot from sheared inputs were evaluated to illustrate that the chromatin was sheared to fragments between 200bp to 1kb and that the amounts of input DNA for the subsequent ChIP assays were equal. **C)** Endogenous YB-1 was immunoprecipitated from MDA-MB-231 cells and ChIP was performed using primers to EGFR1b, EGFR2a, EGFR3 (lanes 1-4). The IgY ChIP was also performed as a negative control (lanes 6-9). **D)** input DNA (lanes 1-4) and genomic DNA was amplified with each of the primers to demonstrate their specificity and the production of an expected product. The expected product size for EGFR1b, EGFR2a, EGFR2b and EGFR3 was 191 bp, 510 bp, 530 bp, and 493 bp (Lanes 10-13), respectively. The no template control was also tested (lane 5-8).

[0025] Figure 3: Over-expression of YB-1 but not YB-1(A102) results in induction of Her-2 and EGFR.

A) The stable expression of Flag:YB-1 increased levels of Her-2 and EGFR protein while the mutant Flag:YB-1(A102) did not. Proteins were isolated from

cells stably expressing either the empty vector (EV), Flag:YB-1, or Flag:YB-1(A102). The relative levels of the transgenes were assessed using an antibody to Flag and YB-1. Vinculin was examined to ensure that the samples were equally loaded. **B)** Expression of Flag:YB-1 increases EGFR mRNA and **C)** HER2 mRNA. The mRNA was harvested from cells growing in log phase, reverse transcribed and then amplified for Her-2 or EGFR expression by qRT-PCR. Each cell line was evaluated in quadruplicates and the data were normalized to TBP. The relative level of induction was compared to the MCF-7(EV) cell line. **D)** Cells that over-express YB-1 are more responsive to EGF stimulation. MCF-7 cells expressing the empty vector (EV), Flag:YB-1, or Flag:YB-1(A102) were serum starved for 24 hrs then stimulated with EGF (40 ng/ml) for 30 min. The cell lines were compared for relative amounts of P-ERK1/2. Total Erk1/2 was included as a control for sample loading.

[0026] Figure 4: Small interfering RNA targeting YB-1 results in loss of EGFR and Her-2 expression. **A)** MCF-7/Flag:YB-1 cells were transfected with siRNA targeting YB-1 for 96 hrs. Compared to the empty vector (lane 1), the targeting vector silenced YB-1 (lanes 2, siYB-1#1, approx. ~50%; lane3, siYB-1#2, approx. ~70%). Knocking down YB-1 inhibited the expression of EGFR (middle panel) but had no effect on the housekeeping protein actin (bottom panel). SiYB-1#1 and siYB-1#2 differ in the amount of lipofectamine used (12.5 and 25 ul respectively), both were transfected with 0.8 ug of pSuperDuper targeting YB-1. **B)** Inhibition of YB-1 using siRNA (top panel) also correlated with a loss of Her-2 protein expression (middle panel) but did not suppress actin (bottom panel).

[0027] Figure 5: Cell permeable peptide targeting YB-1 inhibits tumor growth. MDA-MB-453 or SUM149 cells were plated at a density of 1000 cells/well in a 96-well plate and allowed to attach overnight. The next day, the cells were treated with increasing concentration of the peptide (12.5-50 uM) and tumour cell growth was measured after one week using the MTT assay.

[0028] Figure 6: YB-1 binds to the HER2 promoter

A) Schematic representation of potential YB-1 binding sites and the location of the primers used for ChIP. **B)** MCF-1(Flag:YB-1) and MCF- 7(Flag:YB-

1:A102) cells were subjected to ChIP and the DNA was amplified for Her-2.

5 Flag:YB-1 bound to the Her-2 promoter (lane 1); however the Flag:YB-

1(A102) mutant did not bind effectively (lane 2) and was similar to background amplification with control IgG (lane 3). **C)** YB-1:DNA complexes were isolated

from MDA-MB-453 cells and amplified for Her-2 to confirm endogenous YB-1 binding. YB-1 bound to the Her-2 promoter (lane 2) whereas the IgY negative

10 control did not (lane 3). The sheared input DNA served as a positive control (lane 4).

[0029] Figure 7: YB-1 binding to the EGFR promoter is inhibited with a peptide SEQ ID NO: 11.

15 A) We demonstrate that nuclear proteins from breast cancer cells bind to the EGFR promoter (lane 2) compared to the unbound biotin labeled oligonucleotide (lane 1). Binding was specific because cold competitive oligonucleotides to the same sequence diminished binding (lane 3). The

transcription factor responsible for binding was YB-1 because introducing
20 either 1 or 2 ug (lanes 4-5) cause a super-shift in the binding product. B) The

nuclear extracts from the breast cancer cells were pre-incubated with the cell permeable peptide. YB-1 bound with high affinity in the absence of the inhibitor (DMSO control lane 1) whereas incubation with the cold competitive inhibitor (lane 2), 25 or 50 uM of the peptide blocked binding (lanes 3-4).

25 **[0030] Figure 8 Cell permeable peptide inhibiting YB-1**

phosphorylation inhibits growth of prostate tumor cells. PC3 cells were plated at a density of 1000 cells/well in a 96 well plate and allowed to attach overnight. The next day, the cells were treated with increasing concentration
30 of a peptide inhibitor of YB-1 phosphorylation (12.5-50 uM) and tumour cell growth was measured after one week using the MTT assay. Cell treatment indicated on X-axis, % Absorbance at 490 nm on Y axis.

5 **[0031]** **Figure 9** is a bar graph illustrating data that shows that a YB-1 phosphorylation inhibiting peptide does not affect the viability of primary, normal mammary EpCAM+ cells. Cell viability was measured using MTS assay after 72-hour exposure of two different patient samples to either a scrambled control peptide or the YB-1 specific peptide.

10 **[0032]** **Figure 10** illustrates that a YB-1 phosphorylation inhibiting peptide is more toxic to a breast cancer cell line than to an immortalized line of "normal" breast epithelial cells.

DETAILED DESCRIPTION

15 **[0033]** In the description that follows, a number of terms are used extensively, the following definitions are provided to facilitate understanding of the invention.

20 **[0034]** The terms 'peptide', 'polypeptide' and protein' may be used interchangeably, and refer to a compound comprised of at least two amino acid residues covalently linked by peptide bonds or modified peptide bonds, for example peptide isosteres (modified peptide bonds) that may provide additional desired properties to the peptide, such as increased half-life. A peptide may comprise at least two amino acids. The amino acids comprising a peptide or protein described herein may also be modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Modifications can occur anywhere in a peptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It is understood that the same type of modification may be present in the same or varying degrees at several sites in a given peptide.

30 **[0035]** Examples of modifications to peptides may include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent

attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer- RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, *Proteins-Structure and Molecular Properties*, 2nd ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold F, *Posttranslational Protein Modifications: Perspectives and Prospects*, pgs. 1-12 in *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, ed., Academic Press, New York, 1983; Seifter et al., *Analysis for protein modifications and nonprotein cofactors*, *Meth. Enzymol.* (1990) 182: 626-646 and Rattan et al. (1992), *Protein Synthesis: Posttranslational Modifications and Aging*, " *Ann NY Acad Sci* 663: 48-62.

[0036] Amino acid or nucleic acid sequence similarity or identity may be computed by using the BLASTP and TBLASTN programs which employ the BLAST (basic local alignment search tool) 2.0 algorithm. Techniques for computing amino acid sequence similarity or identity are well known to those skilled in the art, and the use of the BLAST algorithm is described in *ALTSCHUL et al. 1990, J Mol. Biol.* 215: 403- 410 and *ALTSCHUL et al. (1997), Nucleic Acids Res.* 25: 3389-3402.

[0037] Two nucleic acid or protein sequences are considered substantially identical if, when optimally aligned, they share at least about 70% sequence identity. In alternative embodiments, sequence identity may for example be at least 75%, at least 90% or at least 95%. Optimal alignment of sequences for comparisons of identity may be conducted using a variety of algorithms, such as the local homology algorithm of Smith and Waterman, 1981, *Adv. Appl.*

Math 2: 482, the homology alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Natl. Acad. Sci. USA* 85: 2444, and the computerised implementations of these algorithms (such as GAP, BESTFIT, FASTA and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI, U.S.A.). Sequence alignment may also be carried out using the BLAST algorithm, described in Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-10 (using the published default settings). Software for performing BLAST analysis may be available through the National Center for Biotechnology Information (through the internet at ncbi.nlm.nih.gov/). The BLAST algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold. Initial neighbourhood word hits act as seeds for initiating searches to find longer HSPs. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extension of the word hits in each direction is halted when the following parameters are met: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST programs may use as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (Henikoff and Henikoff, 1992, *Proc. Natl. Acad. Sci. USA* 89: 10915-10919) alignments (B) of 50, expectation (E) of 10 (which may be changed in alternative embodiments to 1 or 0.1 or 0.01 or 0.001 or 0.0001; although E values much higher than 0.1 may not identify functionally similar sequences, it is useful to examine hits with lower significance, E values between 0.1 and 10, for short regions of similarity), M=5, N=4, for nucleic acids a comparison of both strands. For protein comparisons, BLASTP may be used with defaults as follows: G=11 (cost to

open a gap); E=1 (cost to extend a gap); E=10 (expectation value, at this setting, 10 hits with scores equal to or better than the defined alignment score, S, are expected to occur by chance in a database of the same size as the one being searched; the E value can be increased or decreased to alter the stringency of the search.); and W=3 (word size, default is 11 for BLASTN, 3 for other blast programs).

[0038] The BLOSUM matrix assigns a probability score for each position in an alignment that is based on the frequency with which that substitution is known to occur among consensus blocks within related proteins. The BLOSUM62 (gap existence cost = 11; per residue gap cost = 1; lambda ratio = 0.85) substitution matrix is used by default in BLAST 2.0. A variety of other matrices may be used as alternatives to BLOSUM62, including: PAM30 (9,1,0.87); PAM70 (10,1,0.87) BLOSUM80 (10,1,0.87); BLOSUM62 (11,1,0.82) and BLOSUM45 (14,2,0.87), the latter of which is set out below. One measure of the statistical similarity between two sequences using the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. In alternative embodiments of the invention, nucleotide or amino acid sequences are considered substantially identical if the smallest sum probability in a comparison of the test sequences is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

hydrogen bonding through the hydroxyl group on the aromatic side chain. Neutral, aliphatic amino acids are generally nonpolar and hydrophobic. Examples of neutral amino acids include alanine, valine, leucine, isoleucine and methionine. An amino acid may be described by more than one
5 descriptive category. Amino acids sharing a common descriptive category may be substitutable for each other in a peptide.

[0040] In accordance with various aspects of the invention, peptides or peptidic sequences, are provided having a sequence derived from the
10 sequence of the Ser102 phosphorylation site of YB-1. That site has the sequence PRKYLRSVG, encompassing an Akt phosphorylation consensus sequence of RXXXRS. This sequence differs from the typical consensus sequence for Akt phosphorylation, which is RXRXXS/T. Accordingly, the distinct phosphorylation consensus sequence of the invention provides a YB-1
15 selective Akt phosphorylation site.

[0041] In one aspect of the invention, YB-1 phosphorylation inhibitors may include peptides that differ from a portion of the native YB-1 phosphorylation site by conservative amino acid substitutions. As used herein, the term
20 "conserved amino acid substitutions" refers to the substitution of one amino acid for another at a given location in the peptide, where the substitution can be made without loss of function. In making such changes, substitutions of like amino acid residues can be made, for example, on the basis of relative similarity of side-chain substituents, for example, their size, charge,
25 hydrophobicity, hydrophilicity, and the like, and such substitutions may be assayed for their effect on the function of the peptide by routine testing.

[0042] In some embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar
30 hydrophilicity value (e.g., within a value of plus or minus 2.0), where the following hydrophilicity values are assigned to amino acid residues (as detailed in United States Patent No. 4,554,101, incorporated herein by

reference): Arg (+3.0); Lys (+3.0); Asp (+3.0); Glu (+3.0); Ser (+0.3); Asn (+0.2); Gln (+0.2); Gly (0); Pro (-0.5); Thr (-0.4); Ala (-0.5); His (-0.5); Cys (-1.0); Met (-1.3); Val (-1.5); Leu (-1.8); Ile (-1.8); Tyr (-2.3); Phe (-2.5); and Trp (-3.4).

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[0043] In alternative embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another having a similar hydropathic index (e.g., within a value of plus or minus 2.0). In such embodiments, each amino acid residue may be assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics, as follows: Ile (+4.5); Val (+4.2); Leu (+3.8); Phe (+2.8); Cys (+2.5); Met (+1.9); Ala (+1.8); Gly (-0.4); Thr (-0.7); Ser (-0.8); Trp (-0.9); Tyr (-1.3); Pro (-1.6); His (-3.2); Glu (-3.5); Gln (-3.5); Asp (-3.5); Asn (-3.5); Lys (-3.9); and Arg (-4.5).

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[0044] In alternative embodiments, conserved amino acid substitutions may be made where an amino acid residue is substituted for another in the same class, where the amino acids are divided into non-polar, acidic, basic and neutral classes, as follows: non-polar: Ala, Val, Leu, Ile, Phe, Trp, Pro, Met; acidic: Asp, Glu; basic: Lys, Arg, His; neutral: Gly, Ser, Thr, Cys, Asn, Gln, Tyr.

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[0045] For example, the invention provides YB-1 phosphorylation inhibitors comprising a contiguous sequence of at least nine residues of the formula:

$$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12}$$

25

wherein:

X_1 is Z or N or D or H or S or X

X_2 is Z or N or D or H or S or X

X_3 is Z or P

X_4 is R

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X_5 is K or R or E or Q or X

X_6 is Y or F or W or X

X_7 is L or M or I or V or F or X

X_8 is R
 X_9 is S
 X_{10} is Z or V or I or L or M or X
 X_{11} is Z or G or X
 X_{12} is Z or D or N or E or X

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in which

X = any amino acid or amino acid analogue,
 Z = not present.

10 **[0046]** In alternative embodiments, the YB-1 phosphorylation inhibitor of the foregoing formula may have:

X_1 is Z or N or D
 X_2 is Z or N or D
 X_3 is Z or P
 X_4 is R
 X_5 is K or R
 X_6 is Y or F or W
 X_7 is L or M or I
 X_8 is R
 X_9 is S
 X_{10} is Z or V or I
 X_{11} is Z or G
 X_{12} is Z or D or N or E.

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25 **[0047]** In accordance with this aspect of the invention, the following matrix sets out sequence variants of the invention, encompassing the forging sequences, in which a number of the residues of the Ser102 phosphorylation site of YB-1 may be replaced with conservative amino acid substitutions, in which these substitutions have positive (favourable) substitution scores on the
 30 BLOSUM 45 matrix (set out above).

X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	X_9	X_{10}	X_{11}	X_{12}
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N ⁹⁴	N ⁹⁵	P ⁹⁶	R ⁹⁷	K ⁹⁸	Y ⁹⁹	L ¹⁰⁰	R ¹⁰¹	S ¹⁰²	V ¹⁰³	G ¹⁰⁴	D ¹⁰⁵
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	X	X	X	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	F	M	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	W	M	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	F	I	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	W	I	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	F	V	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	W	V	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	F	F	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	R	W	F	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	F	M	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X

Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	W	M	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	F	I	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	W	I	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	F	V	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	W	V	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	F	F	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	E	W	F	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	F	M	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	W	M	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	F	I	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	W	I	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X

S/X	S/X								/X		X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	F	V	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	W	V	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	F	F	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X
Z/N/ D/H/ S/X	Z/N/ D/H/ S/X	Z/P	R	Q	W	F	R	S	Z/V/I /L/M /X	Z/G/ X	Z/D/ N/E/ X

Where X = any amino acid, Z = not present, and alternative amino acids are indicated by a "/"

[0048] Nomenclature used to describe the peptide compounds of the present invention follows the conventional practice where the amino group is presented to the left and the carboxy group to the right of each amino acid residue. In the sequences representing selected specific embodiments of the present invention, the amino- and carboxy-terminal groups, although not specifically shown, will be understood to be in the form they would assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue may be generally represented by a one-letter or three-letter designation, corresponding to the trivial name of the amino acid, in accordance with the following Table 2:

[0049] Table 2 Nomenclature and abbreviations of the 20 standard L-amino acids commonly found in naturally occurring peptides.

Full name	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A

Cysteine	Cys	C
Aspartic acid	Asp	D
Glutamic acid	Glu	E
Phenylalanine	Phe	F
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Lysine	Lys	K
Leucine	Leu	L
Methionine	Met	M
Asparagine	Asp	N
Proline	Pro	P
Glutamine	Gln	Q
Arginine	Arg	R
Serine	Ser	S
Threonine	Thr	T
Valine	Val	V
Tryptophan	Trp	W
Tyrosine	Tyr	Y

[0050] Amino acids comprising the peptides described herein will be understood to be in the L- or D- configuration. In peptides and peptidomimetics of the present invention, D-amino acids may be substitutable for L-amino acids.

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[0051] Amino acids contained within the peptides of the present invention, and particularly at the carboxy-or amino-terminus, may be modified by methylation, amidation, acetylation or substitution with other chemical groups

which may change the circulating half-life of the peptide without adversely affecting their biological activity. Additionally, a disulfide linkage may be present or absent in the peptides of the invention.

5 **[0052]** Nonstandard amino acids may occur in nature, and may or may not be genetically encoded. Examples of genetically encoded nonstandard amino acids include selenocysteine, sometimes incorporated into some proteins at a UGA codon, which may normally be a stop codon, or pyrrolysine, sometimes incorporated into some proteins at a UAG codon, which may normally be a stop codon. Some nonstandard amino acids that are not genetically encoded may result from modification of standard amino acids already incorporated in a peptide, or may be metabolic intermediates or precursors, for example. Examples of nonstandard amino acids include 4-hydroxyproline, 5-hydroxylysine, 6-N-methyllysine, gamma-carboxyglutamate, desmosine, 10 selenocysteine, ornithine, citrulline, lanthionine, 1-aminocyclopropane-1-carboxylic acid, gamma-aminobutyric acid, carnitine, sarcosine, or N-formylmethionine. Synthetic variants of standard and non-standard amino acids are also known and may include chemically derivatized amino acids, amino acids labeled for identification or tracking, or amino acids with a variety of side groups on the alpha carbon. Examples of such side groups are known in the art and may include aliphatic, single aromatic, polycyclic aromatic, heterocyclic, heteronuclear, amino, alkylamino, carboxyl, carboxamide, carboxyl ester, guanidine, amidine, hydroxyl, alkoxy, mercapto-, 15 alkylmercapto-, or other heteroatom-containing side chains. Other synthetic amino acids may include alpha-imino acids, non-alpha amino acids such as beta-amino acids, des-carboxy or des-amino acids. Synthetic variants of amino acids may be synthesized using general methods known in the art, or may be purchased from commercial suppliers, for example RSP Amino Acids LLC (Shirley, MA).

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[0053] A peptidomimetic is a compound comprising non-peptidic structural elements that mimics the biological action of a parent peptide. A

peptidomimetic may not have classical peptide characteristics such as an enzymatically scissile peptidic bond. A parent peptide may initially be identified as a binding sequence or phosphorylation site on a protein of interest, or may be a naturally occurring peptide, for example a peptide hormone. Assays to identify peptidomimetics may include a parent peptide as a positive control for comparison purposes, when screening a library, such as a peptidomimetic library. A peptidomimetic library is a library of compounds that may have biological activity similar to that of a parent peptide.

[0054] The term 'antibody' as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, or humanized antibodies, as well as Fab or F(ab)² fragments, including the products of an Fab or other immunoglobulin expression library.

[0055] Peptides according to one embodiment of the invention may include peptides comprising the amino acid sequence PRKYLRSVG (SEQ ID NO: 12). Other peptides according to other embodiments of the invention may include peptides having a substantially similar sequence to that of SEQ ID NO: 12. Such peptides may be in isolation, or may be linked to or in combination with tracer compounds, protein translocation sequences, liposomes, carbohydrate carriers, polymeric carriers or other agents or excipients as will be apparent to one of skill in the art. In an alternate embodiment, such peptides may comprise a medicament, wherein such peptides may be present in a pharmacologically effective amount.

[0056] The term 'medicament' as used herein, a "medicament" refers to a composition that may be administered to a patient or test subject and is capable of producing an effect in the patient or test subject. The effect may be chemical, biological or physical, and the patient or test subject may be human, or a non-human animal, such as a rodent or transgenic mouse, or a dog, cat, cow, sheep, horse, hamster, guinea pig, rabbit or pig. The

medicament may be comprised of the effective chemical entity alone or in combination with a pharmaceutically acceptable excipient.

[0057] The term 'pharmaceutically acceptable excipient' may include any and all solvents, dispersion media, coatings, antibacterial, antimicrobial or antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. An excipient may be suitable for intravenous, intraperitoneal, intramuscular, subcutaneous, intrathecal or oral administration. An excipient may include sterile aqueous solutions or dispersions for extemporaneous preparation of sterile injectable solutions or dispersion. Use of such media for preparation of medicaments is known in the art.

[0058] Delivery of bioactive molecules such as peptides, to a cell or cells in a reasonably efficient manner may require more than just the 'dumping' of the naked peptide onto the cell, or administering the naked peptide into the patient or test subject. Agents that enable delivery of bioactive molecules into cells in a suitable manner so as to provide an effective amount, such as a pharmacologically effective amount are known in the art, and are described in, for example, *DIETZ et al 2004. Mol Cell. Neurosci 27:85-131*. Examples of such agents include liposomes, antibodies or receptor ligands that may be coupled to the bioactive molecule, viral vectors, and protein transduction domains (PTD). Examples of PTDs include *Antennapedia* homeodomain (*PEREZ et al 1992 J. Cell Sci 102:717-722*), transportan (*POOGA et al 1998 FASEB J 12: 67-77*), the translocation domains of diphtheria toxin (*STENMARK et al 1991 J Cell Biol 113:1025-1032*; *WIEDLOCHA et al 1994 Cell 76:1039-1051*), anthrax toxin (*BALLARD et al 1998 Infect. Immun 66:615-619*; *BLANKE et al 1996 Proc Natl Acad Sci 93: 8437-8442*) and *Pseudomonas* exotoxin A (*PRIOR et al 1992 Biochemistry 31:3555-3559*), protegrin derivatives such as dermaseptin S4 (*HARITON-GAZAL et al 2002 Biochemistry 41:9208-9214*), HSV-1 VP22 (*DILBER et al 1999 Gene Ther. 6:12-21*), PEP-1 (*MORRIS et al 2001 Nature Biotechnol 19:1173-1176*), basic

peptides such as poly-L and poly-D-lysine (*WOLFERT et al 1996 Gene Ther.* 3:269-273 ; *RYSER et al 1980 Cancer* 45:1207-1211; *SHEN et al 1978 Proc Natl Acad Sci* 75:1872-1876), HSP70 (*FUJIHARA et al 1999 EMBO J* 18:411-419) and HIV-TAT (*DEMARCHI et al 1996 J Virol* 70:4427-4437). Other
5 examples and related details of such protein transduction domains are described in *DIETZ, supra* and references therein.

[0059] The term 'pharmacologically effective amount' of a medicament may refer to an amount of a medicament present in such a concentration to
10 result in a therapeutic level of drug delivered over the term that the drug is used and producing a chemical, biological or physical effect in the patient or test subject. The pharmacologically effective amount may be dependent on the mode of delivery, site of administration of the medicament, time period of the dosage, age, weight, general health, sex or diet of the patient or test
15 subject receiving the medicament.

[0060] As used herein, the term "cancer" refers to a proliferative disorder caused or characterized by the proliferation of cells which have lost susceptibility to normal growth control. The term cancer, as used in the
20 present application, includes tumors and any other proliferative disorders. Cancers of the same tissue type usually originate in the same tissue, and may be divided into different subtypes based on their biological characteristics. Four general categories of cancers are carcinoma (epithelial tissue derived), sarcoma (connective tissue or mesodermal derived), leukemia (blood-forming
25 tissue derived) and lymphoma (lymph tissue derived). Over 200 different types of cancers are known, and every organ and tissue of the body may be affected. Specific examples of cancers that do not limit the definition of cancer may include melanoma, leukemia, astrocytoma, glioblastoma, retinoblastoma, lymphoma, glioma, Hodgkins' lymphoma and chronic
30 lymphocyte leukemia. Examples of organs and tissues that may be affected by various cancers include pancreas, breast, thyroid, ovary, uterus, testis, prostate, thyroid, pituitary gland, adrenal gland, kidney, stomach, esophagus

or rectum, head and neck, bone, nervous system, skin, blood, nasopharyngeal tissue, lung, urinary tract, cervix, vagina, exocrine glands and endocrine glands. Alternatively, a cancer may be multicentric or of unknown primary site (CUPS).

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[0061] As used herein, a 'cancerous cell' refers to a cell that has undergone a transformation event and whose growth is no longer regulated to the same extent as before said transformation event. A tumor refers to a collection of cancerous cells, often found as a solid or semi-solid lump in or on the tissue or a patient or test subject.

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[0062] A cancer or cancerous cell may be described as "sensitive to" or "resistant to" a given therapeutic regimen or chemotherapeutic agent based on the ability of the regimen to kill cancer cells or decrease tumor size, reduce overall cancer growth (i.e. through reduction of angiogenesis), and/or inhibit metastasis. Cancer cells that are resistant to a therapeutic regimen may not respond to the regimen and may continue to proliferate. Cancer cells that are sensitive to a therapeutic regimen may respond to the regimen resulting in cell death, a reduction in tumor size, reduced overall growth or inhibition of metastasis. Monitoring of a response may be accomplished by numerous pathological, clinical and imaging methods as described herein and known to persons of skill in the art.

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[0063] A common theme for a chemotherapeutic agent or combination of agents is to induce death of the cancerous cells. For example, DNA adducts such as nitrosoureas, busulfan, thiotepa, chlorambucil, cisplatin, mitomycin, procarbazine, or dacarbazine slow the growth of the cancerous cell by forcing the replicating cell to repair the damaged DNA before the M-phase of the cell cycle, or may by themselves cause sufficient damage to trigger apoptosis of the cancerous cell. Other events such as gene expression or transcription, protein translation, or methylation of the replicated DNA, for example, may also be interfered with by the varied arsenal of

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chemotherapeutic agents available to the clinician and help to trigger apoptotic processes within the cancerous cells. Alternately, a chemotherapeutic agent may enable the cancerous cell to be killed by aspects of the patient or test subject's humoral or acquired immune system, for example, the complement cascade or lymphocyte attack.

[0064] A cancerous cell resistant to a chemotherapeutic agent or combination of agents may fight for its survival by actively transporting the drug out of the cell for example, by overexpression of the ABC transporter MDR1 p-glycoprotein (*FORD et al 1993. Cytotechnol. 12:171-212*) or acquiring 'counter-mutations' to counteract the drugs. For example, mutations in the DNA repair enzymes that affect the ability to detect damage to the cells' DNA may enable replication of the damaged DNA and permit the cancerous cells to continue replicating, enlarging the tumor. As mutations accumulate, other regulatory points that would otherwise act in a normal cell cycle cease to function, and the cycle of unregulated growth cascades. Another aspect of chemotherapeutic resistance involves the tumor cells' avoidance of apoptosis. A host organism's normal response to dysregulated cell growth is to initiate apoptosis and eliminate the defective cell before the cascade into uncontrolled replication begins, however this may be subverted by a cancerous cell, for example, by disruption of signal transduction events, loss of adhesion dependence or contact inhibition in the cancerous cell, or loss of apoptosis-promoting factors, often considered 'tumor suppressors', for example p53, BRCA1 or RB. The importance of this sensitivity to apoptosis in the treatment of cancer is supported by recent evidence indicating that the selectivity of chemotherapy for the relatively few tumors ever cured solely by drugs depends, to a large extent, upon their easy susceptibility to undergo apoptosis (*JOHNSTONE et al., 2002. Cell. 108(2):153-64*)

[0065] As used herein, a 'therapeutic regimen refers to a chemotherapeutic regimen or a radiotherapy regimen, or a combination thereof.

[0066] As used herein, a “chemotherapeutic regimen” or “chemotherapy” refers to the use of at least one chemotherapy agent to destroy cancerous cells. There are a myriad of such chemotherapy agents available to a clinician. Chemotherapy agents may be administered to a subject in a single bolus dose, or may be administered in smaller doses over time. A single chemotherapeutic agent may be used (single-agent therapy) or more than one agent may be used in combination (combination therapy). Chemotherapy may be used alone to treat some types of cancer. Alternatively, chemotherapy may be used in combination with other types of treatment, for example, radiotherapy or alternative therapies (for example immunotherapy) as described herein. Additionally, a chemosensitizer may be administered as a combination therapy with a chemotherapy agent.

[0067] As used herein, a ‘chemotherapeutic agent’ refers to a medicament that may be used to treat cancer, and generally has the ability to kill cancerous cells directly. Examples of chemotherapeutic agents include alkylating agents, antimetabolites, natural products, hormones and antagonists, and miscellaneous agents. Examples of alternate names are indicated in brackets. Examples of alkylating agents include nitrogen mustards such as mechlorethamine, cyclophosphamide, ifosfamide, melphalan (L-sarcolysin) and chlorambucil; ethylenimines and methylmelamines such as hexamethylmelamine and thiotepa; alkyl sulfonates such as busulfan; nitrosoureas such as carmustine (BCNU), semustine (methyl-CCNU), lomustine (CCNU) and streptozocin (streptozotocin); DNA synthesis antagonists such as estramustine phosphate; and triazines such as dacarbazine (DTIC, dimethyl-triazenoimidazolecarboxamide) and temozolomide. Examples of antimetabolites include folic acid analogs such as methotrexate (amethopterin); pyrimidine analogs such as fluorouracil (5-fluorouracil, 5-FU, 5FU), floxuridine (fluorodeoxyuridine, FUdR), cytarabine (cytosine arabinoside) and gemcitabine; purine analogs such as mercaptopurine (6-mercaptopurine, 6-MP), thioguanine (6-thioguanine, TG)

and pentostatin (2'-deoxycoformycin, deoxycoformycin), cladribine and fludarabine; and topoisomerase inhibitors such as amsacrine. Examples of natural products include vinca alkaloids such as vinblastine (VLB) and vincristine; taxanes such as paclitaxel and docetaxel (Taxotere);

5 epipodophyllotoxins such as etoposide and teniposide; camptothecins such as topotecan and irinotecan; antibiotics such as dactinomycin (actinomycin D), daunorubicin (daunomycin, rubidomycin), doxorubicin, bleomycin, mitomycin (mitomycin C), idarubicin, epirubicin; enzymes such as L-asparaginase; and biological response modifiers such as interferon alpha and

10 interleukin 2. Examples of hormones and antagonists include luteinising releasing hormone agonists such as buserelin; adrenocorticosteroids such as prednisone and related preparations; progestins such as hydroxyprogesterone caproate, medroxyprogesterone acetate and megestrol acetate; estrogens such as diethylstilbestrol and ethinyl estradiol and related

15 preparations; estrogen antagonists such as tamoxifen and anastrozole; androgens such as testosterone propionate and fluoxymesterone and related preparations; androgen antagonists such as flutamide and bicalutamide; and gonadotropin-releasing hormone analogs such as leuprolide. Examples of

20 miscellaneous agents include thalidomide; platinum coordination complexes such as cisplatin (*cis*-DDP), oxaliplatin and carboplatin; anthracenediones such as mitoxantrone; substituted ureas such as hydroxyurea; methylhydrazine derivatives such as procarbazine (N-methylhydrazine, MIH); adrenocortical suppressants such as mitotane (o,p'-DDD) and aminoglutethimide; RXR agonists such as bexarotene; and tyrosine kinase

25 inhibitors such as imatinib. Alternate names and trade-names of these and additional examples of chemotherapeutic agents, and their methods of use including dosing and administration regimens, will be known to a physician versed in the art, and may be found in, for example "The Pharmacological basis of therapeutics", 10th edition. HARDMAN HG., LIMBIRD LE. editors.

30 McGraw-Hill, New York, and in "Clinical Oncology", 3rd edition. Churchill Livingstone/ Elsevier Press, 2004. ABELOFF, MD. editor.

[0068] As used herein, a 'chemosensitizer' refers to a medicament that may enhance the therapeutic effect of a chemotherapeutic agent or radiotherapy treatment and therefore improve efficacy of such treatment or agent. Examples of chemosensitizers may be found in Table 3. The sensitivity or resistance of a tumor or cancerous cell to treatment may also be measured in an animal, such as a human or rodent, by measuring the tumor size reduction over a period of time. For example, 6 months for a human and 4-6 weeks for a mouse. A composition or a method of treatment may sensitize a tumor or cancerous cell's response to a therapeutic treatment if the increase in treatment sensitivity or the reduction in resistance is 10% or more, for example, 30%, 40%, 50%, 60%, 70%, 80%, or more, to 2- fold, 3-fold, 4-fold, 5-fold, 10-fold, 15-fold, 20-fold or more, compared to treatment sensitivity or resistance in the absence of such composition or method. The determination of sensitivity or resistance to a therapeutic treatment is routine in the art and within the skill of an ordinarily skilled clinician.

Table 3 Radio- and Chemo- sensitizing drugs.

Source	Drug	Mode of Action
Angelopharm	Lonidamine	Protein Synthesis Inhibitor
Biolitec Pharma	Temoporfin	Photosensitizer – promotes radical formation
Meiji Seika	Talaporfin sodium	Photosensitizer – promotes radical formation
Abbott	ABT-472	ADP ribose polymerase 1 inhibitor
Apogee	Pgp Inhibitors	Inhibit Pgp mediated drug efflux
Berkeley Labs	β -1 integrin antagonists	β -1 integrin antagonists, for sensitizing cancer to ionizing radiation

BioFocus	Chk-1 kinase inhibitors	Chk-1 kinase inhibitors enhance efficacy of chemotherapy and radiotherapy
ChemGenex Pharmaceuticals	CGX-6001	?
Hana Biosciences	IPdR	DNA Synthesis Inhibitor
IC-MedTech	apatone	Apoptosis agonists-induces oxidative stress
Inex Pharmaceuticals	INX-0167	Immunostimulant-activates Killer T-cells
KuDos	1,3-dipropyl-7-methylxanthine, KU-0064, KU-0687	DNA repair enzyme inhibitor, ADP ribose polymerase inhibitor
OSI Pharmaceuticals	CYP26 inhibitors	Inhibits metabolism of Chemotherapeutic agents
Peregrine Pharmaceuticals	IL-2 Fragment (VEA)	Membrane permeability enhancer
Quintessence Biosciences	EVade ribonucleases	Ribonuclease stimulant
Chiron	MPI-5020	
Allos Therapeutics	efaproxiral sodium	allosteric modifier of haemoglobin. It increases the amount of oxygen released by haemoglobin, enhancing the efficacy of the radiotherapy
Adherex	Exherin	N-cadherin inhibitor
GSK	elacridar	P glycoprotein inhibitor
Light Sciences	LS-216	Photosensitizer-Radical formation agonist

Pfizer (licensed to Cancer Research Technology in UK)	PARP inhibitors, Agouron	ADP ribose polymerase inhibitor
Adventrx	CoFactor	Thymidylate synthase inhibitor-use in colorectal cancer
Cancer Research Technology	PaTrin-2	O6-alkylguanine-DNA alkyltransferase inhibitor
Pharmacyclics	lutetium texaphyrin	Photosensitizer
Pola	doranidazole	Hypoxic Cell Sensitizer-Apoptosis agonist
RESprotect	RP-101	targeted to prevent cells from developing resistance to chemotherapy (pancreatic cancer)
Xenova	tariquidar	PgP inhibitor
Yeda	Pd-bacteriopheophorbide	photosensitizing agent
Pharmacyclics	motexafin gadolinium (Xcytrin™)	Radiosensitizer-radical formation agonist
Schering	MS209	PgP Inhibitor
SRI International	tirapazamine	Reducing agent

WellStat	triacetyluridine	a prodrug of the nucleoside uridine, to enable higher dosage of 5-FU to be administered to cancer patients
YM Biosciences	tesmilifene hydrochloride	Histamine Antagonist

[0069] As used herein, the term “radiotherapeutic regimen” or “radiotherapy” refers to the administration of radiation to kill cancerous cells.

5 Radiation interacts with various molecules within the cell, but the primary target, which results in cell death is the deoxyribonucleic acid (DNA).

However, radiotherapy often also results in damage to the cellular and nuclear membranes and other organelles. DNA damage usually involves single and double strand breaks in the sugar-phosphate backbone. Furthermore, there can be cross-linking of DNA and proteins, which can disrupt cell function.

10 Depending on the radiation type, the mechanism of DNA damage may vary as does the relative biologic effectiveness. For example, heavy particles (i.e. protons, neutrons) damage DNA directly and have a greater relative biologic effectiveness. Electromagnetic radiation results in indirect ionization acting

15 through short-lived, hydroxyl free radicals produced primarily by the ionization of cellular water. Clinical applications of radiation consist of external beam radiation (from an outside source) and brachytherapy (using a source of radiation implanted or inserted into the patient). External beam radiation consists of X-rays and/or gamma rays, while brachytherapy employs
20 radioactive nuclei that decay and emit alpha particles, or beta particles along with a gamma ray.

[0070] Radiotherapy may further be used in combination chemotherapy, with the chemotherapeutic agent acting as a radiosensitizer. The specific
25 choice of radiotherapy suited to an individual patient may be determined by a

physician or oncologist, taking into consideration the tissue and stage of the cancer. Examples of radiotherapy approaches to various cancers may be found in, for example "Clinical Oncology", 3rd edition. Churchill Livingstone/Elsevier Press, 2004. ABELOFF, MD. editor.

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[0071] As used herein, the term "alternative therapeutic regimen" or "alternative therapy" may include for example, receptor tyrosine kinase inhibitors (for example Iressa™ (gefitinib), Tarceva™ (erlotinib), Erbitux™ (cetuximab), imatinib mesilate (Gleevec™), proteosome inhibitors (for example bortezomib, Velcade™); VEGFR2 inhibitors such as PTK787 (ZK222584), aurora kinase inhibitors (for example ZM447439); mammalian target of rapamycin (mTOR) inhibitors, cyclooxygenase-2 (COX-2) inhibitors, rapamycin inhibitors (for example sirolimus, Rapamune™); farnesyltransferase inhibitors (for example tipifarnib, Zarnestra); matrix metalloproteinase inhibitors (for example BAY 12-9566; sulfated polysaccharide tecogalan); angiogenesis inhibitors (for example Avastin™ (bevacizumab); analogues of fumagillin such as TNP-4; carboxyamino triazole; BB-94 and BB-2516; thalidomide; interleukin-12; linomide; peptide fragments; and antibodies to vascular growth factors and vascular growth factor receptors); platelet derived growth factor receptor inhibitors, protein kinase C inhibitors, mitogen-activated kinase inhibitors, mitogen-activated protein kinase kinase inhibitors, Rous sarcoma virus transforming oncogene (SRC) inhibitors, histone deacetylase inhibitors, small hypoxia-inducible factor inhibitors, hedgehog inhibitors, and TGF- β signalling inhibitors. Furthermore, an immunotherapeutic agent would also be considered an alternative therapeutic regimen. For example, serum or gamma globulin containing preformed antibodies; nonspecific immunostimulating adjuvants; active specific immunotherapy; and adoptive immunotherapy. In addition, alternative therapies may include other biological-based chemical entities such as polynucleotides, including antisense molecules, polypeptides, antibodies, gene therapy vectors and the like. Such alternative therapeutics may be administered alone or in combination, or in combination with other therapeutic

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regimens described herein. Alternate names and trade-names of these agents used in alternative therapeutic regimens and additional examples of agents used in alternative therapeutic regimens, and their methods of use including dosing and administration regimens, will be known to a physician versed in the art. Furthermore, methods of use of chemotherapeutic agents and other agents used in alternative therapeutic regimens in combination therapies, including dosing and administration regimens, will also be known to a physician versed in the art.

[0072] A 'YB-1 expressing cancer' or a 'YB-1 expressing cancerous cell' refers to a cancer or cancerous cell that expresses Y-box protein 1 (YB-1). The relative level of YB-1 protein or nucleic acid transcript may be at the same level as a non-cancerous cell, or may be found at a level higher than that in a non-cancerous cell. For example, the level of YB-1 protein or nucleic acid transcript expression may be at least 10% higher than that in a non-cancerous cell. Alternatively, the level of YB-1 protein or nucleic acid transcript expression may be 25%, 50%, 100%, 200% or greater than that in a non-cancerous cell. Levels of expression of specific protein or nucleic acids may be determined using methods known to those of skill in the art, and are described in, for example AUSUBEL *et al.*, Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., 1998; ABELOFF, Clinical Oncology, 3rd edition. Churchill Livingstone/ Elsevier Press, 2004; HARLOW, E and LANE D. Antibodies: A Laboratory Manual. 1988. Cold Spring Harbor Laboratory Press; SAMBROOK J and RUSSELL DW. Molecular cloning: A Laboratory Manual 2001 Cold Spring Harbor Laboratory Press; Harrison's Principles of Internal Medicine 15th ed. BRAUNWALD *et al* eds. McGraw-Hill.

[0073] YB-1 acts as a transcriptional activator of some genes, some of which have key roles in the development and progression of some cancers. For example, expression of YB-1 in some cell lines induces expression of EGFR and HER2. Knocking down YB-1 expression inhibits cell growth in cancer cell lines.

[0074] In one embodiment of the invention, methods of inhibiting expression of YB-1 in a YB-1 expressing cancer or a YB-1 expressing cancerous cell may include contacting the cell or cells with an inhibitor of YB-1 expression, for example an siRNA of YB-1.

[0075] YB-1 transcriptional activation may also be inhibited, and reduce the expression of a gene having YB-1 binding sequences or motifs. Inhibiting the binding of YB-1 to a promoter motif of a YB-1 responsive gene, for example EGFR, inhibits cell growth.

[0076] In another embodiment of the invention, methods of inhibiting YB-1 mediated transcription in a YB-1 expressing cancer or cancerous cell may include contacting the cell or cells with an inhibitor of YB-1 transcriptional activation, or a peptidic sequence that is a YB-1 selective Akt phosphorylation recognition site, for example, a peptide corresponding to SEQ ID NO: 12, or a substantially similar sequence thereof. While a YB-1 selective Akt phosphorylation recognition site may have sequence similarity to a native YB-1 site recognized by Akt, there is no requirement for such recognition to result in phosphorylation of the peptidic sequence in each embodiment. In another example, an inhibitor of YB-1 transcriptional activation may include an antibody or antibody fragment capable of binding to a sequence corresponding to SEQ ID NO: 12.

[0077] It may be advantageous to incorporate aspects of the present invention, for example YB-1 transcriptional inhibitors in combination therapy with known chemotherapeutic agents or alternative therapeutic agents, for example ERBITUX™ (cetuximab) or HERCEPTIN™ (trastuzumab). It is known in the art that a cancerous cell must express EGFR to be sensitive to cetuximab, or express HER2 to be sensitive to trastuzumab, however a subset of cancerous cells expressing either of these receptors are resistant to these therapeutic agents. Combining a YB-1 transcriptional inhibitor, for

example a peptide corresponding to SEQ ID NO: 12 or a substantially similar sequence, with one of these examples of chemotherapeutic agents may sensitize the cancerous cells to the chemotherapeutic agent, or may enable a lower dosage of the chemotherapeutic agent. A YB-1 transcriptional inhibitor used in this manner, for example, may also be described as a chemosensitizer, or if used in combination with radiotherapy, a radiosensitizer.

EXAMPLES

Methods and Materials

Sequences of Peptides and Primers

SEQ ID NO:	Sequence	Name
1	TCGCCGCCAACGCCACAAC	EGFR1b forward
2	ACACGCCCTTACCTTTCTTTTCTCCAG	EGFR1b reverse
3	CCGCGAGTTTCCCTCGCATTCT	EGFR2a forward
4	CCTTCCCCCTTTCCCTTCTTTTGTTTAC	EGFR2a reverse
5	TCCCATTGTCCTTTCTCTAGTTTGTTTC	EGFR2b forward
6	GTCCACCCCATCCCCACTGTTCTTCTC	EGFR2b reverse
7	TTCAGCAAACCCATTCTTCT	EGFR3 forward
8	GCTTCCTGCACACCTGGGCTGAG	EGFR3 reverse
9	AGGGGCTCCAAATAGAATGT	HER-2 forward
10	AATTTGGGAGGAGACAGTCA	HER2

		reverse
11	RQIKIWFQNRRMKWKKPRKYLRSVG	AP-CSD
12	PRKYLRSVG	CSD
13	TTCACACATTGGCTTCAAAGTACCCATGGCTGGTTG CAATAAACAT	EMSA
14	ATGTTTATTGCACAACCAGCCATGGGTACTTTGAAG CCAATGTGTGAA	EMSA

Tumor Tissue Microarray

[0078] Tissue microarrays (TMA) were constructed from 438 cases of breast carcinoma from the archives at Vancouver General Hospital, as described previously (*SUTHERLAND et al 2005. Oncogene 24:4281-4292; MAKRETISOV et al 2003. Hum. Pathol 34:1001-1008*). Duplicate 0.6 mm cores of formalin-fixed paraffin embedded cores were used for array construction. Tissue microarray (TMA) slides were immunostained for EGFR and Her-2 as previously described (*KUCAB et al 2005. Breast Cancer Res 7:R796-R807*). To detect YB-1, the tissues underwent antigen retrieval by heating to 100°C in a citrate buffer for 30 minutes, and the slides were incubated with anti-YB-1 antibody at 1:2000 overnight. YB-1 was detected using the LSAB+ system (Dako, Carpinteria, CA) (*SUTHERLAND, supra*). For interpretation of immunostaining, only cores containing invasive carcinoma were considered interpretable. Results from duplicate cores were combined to give a single result per case. Where there were discordant results for the duplicate cores, the higher result was accepted for that case as described previously (*MAKRETISOV, supra*). For YB-1, tumors showing no staining or weak focal staining were considered to be YB-1 negative, while moderate or intense staining was considered positive. YB-1 immunostaining was predominantly found in the cytoplasm. The statistical analyses were based on the amount of YB-1 expressed in the cytoplasm.

[0079] Tissue microarray slides were digitized using Bliss slide scanner based on Olympus BX61 microscope (Bacus Laboratories, Inc., Lombard, IL). Nine pictures were taken from each core, and the pixel dimensions were tiled to create a composite image. The images were viewed using the WebSlide Browser program (Bacus Laboratories). A Kaplan-Meier curve was generated using a non-parametric method for survival. Survival estimates (log rank and Breslow) are based on the weighted differences between actual and expected numbers of deaths at the observed time points. The log rank test factors in all deaths equally, while Breslow tests give more weight to early deaths. All statistical analyses were performed using the SPSS 11.0 statistical package. Cox regression model is used for multivariate survival analysis. Tumor collection and analyses were performed in accordance with the guidelines established by the Vancouver General Hospital Research Ethics Board.

Cell Lines and Reagents

[0080] The PC-3, MCF-7, MDA-MB-231, MDA-MB-468 and MDA-MB-453 breast cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA). SUM149 cells were obtained from Astrand. JMIT-1 cells were obtained from the BC Cancer Agency (Dr. M. Bally). The C-terminal rabbit polyclonal antisera was produced as previously described (*GIMENEZ-BONAFE supra*). The N-terminal chicken polyclonal antibody was raised against a YB-1 amino-terminus peptide MSSEAETQQPPAA (*BERQUIN supra*). Flag:YB-1 transgenes were detected using a M2 mouse monoclonal antibody to Flag (Sigma, St Louis MO). Flag:YB-1(S102) was mutated to Flag:YB-1(A102) by site directed mutagenesis as previously described (*SUTHERLAND, supra*).

[0081] Cells were transfected using Lipofectamine 2000 (Invitrogen), following manufacturer's instructions

Western Blotting

[0082] Proteins were isolated from MCF-7 cells expressing either the empty vector

(EV), Flag:YB-1 or Flag:YB-1(A102). Cells were 80% confluent when harvested by scraping. Cells were lysed in ELB buffer (5mM Hepes pH 7.4, 150mM NaCl, 1mM EDTA (pH 8), 1% Triton-X 100, 1% deoxycholate, and 0.1% SDS) with protease inhibitors and sheared through a 21gauge needle. Proteins (100 ug/lane) were run on a 12% SDS polyacrylamide gel and transferred to a nitrocellulose membrane at 40V overnight at 4°C. The membrane was then probed with anti-EGFR (1:275, StressGen CSA330) or anti-Her-2 (1:500, Abcam ab 2428) antibodies. Vinculin (1:2000, Sigma Clone Vin 11-5, V4505 antibody) and actin (Sigma) were used as loading controls. MCF-7 cells expressing the various constructs were also serum starved overnight then stimulated with EGF (40 ng/ml) for 30 min. The extracts were then evaluated for activation of signaling through the MAP kinase pathway using P-Erk 1/2 antibody (Thr 202/Tyr 204, 1:1000, Cell Signaling).

Real Time qRT-PCR

[0083] RNA was isolated from MCF-7 cells (transfected with empty vector, Flag:YB-1 or Flag:YB-(A102) grown in log phase. The RNA was reverse transcribed and amplified using Her-2 or EGFR specific primers and probes (Applied Biosystems, Foster City, CA). TATA box binding protein mRNA was measured as a housekeeping gene (Applied Biosystems) as previously described (*OH et al 2002 Neoplasia 4:204-217*). Each sample was analyzed in replicates of four on two separate occasions.

MTT assay for cell growth

[0084] For the MTT assay, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxy-methoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt (MTS) and phenazine methosulfate (PMS), were mixed in the proportions 2:0.92 µg/ml, and 25 µl of the mixture was added to each well. After incubation at 37 °C with 5% CO₂ for 2 h, the absorbance of each well at 490 nm was measured in a

96-well plate reader according to the manufacturer's instructions. Cell viability was expressed as a percentage of control.

Chromatin Immunoprecipitation:

5 **[0085]** ChIP assays were performed using the ChIP-IT kit (Active Motif #101198) according to manufacturer's instructions. Buffers and reagents were provided with the ChIP-IT kits unless otherwise indicated. In brief, MCF7/Flag:YB-1 and MCF7/Flag:YB-1A102 cells were grown on 15-cm plates in phenol-red free RPMI with 5%FBS and 400µg/ml G418. The cells (8x10⁶ cells/150 mm dish, 80% confluent) were fixed for 10min with 1% formaldehyde. After quenching the reaction with glycine for 5min, cells were treated with Lysis Buffer and extracted using a Dounce homogenizer. The nuclei were isolated and chromatin was sonicated in Shearing Buffer using a Cole Parmer Ultrasonic Processor at 25% power with 10 pulses of 20 seconds each and a 30 seconds rest on ice between each pulse to give chromatin fragments between 200bp and 1kb. Chromatin was then pre-cleared with salmon sperm DNA/protein G agarose and incubated with 3µg anti-FLAG M2 monoclonal antibody (Sigma #F3165) overnight at 4°C. Chromatin incubated with 3µg mouse IgG overnight at 4°C was used as a negative control. Salmon sperm DNA/protein G agarose was subsequently incubated with the chromatin and antibody for 1.5hr at 4°C. The immunoprecipitated material was washed twice with ChIP IP Buffer, 5 times with Wash Buffer 1, twice with Wash Buffer 2, and 3 times with Wash Buffer 3. To reverse crosslink and to remove RNA, the immunoprecipitated material was incubated in 200mM NaCl and 10µg RNase A overnight at 65°C. The proteins were then removed by proteinase K treatment, and DNA was purified using the DNA purification minicolumns provided with the kit.

30 **[0086]** Eluted DNA (5 ul) was amplified by PCR with the following primers: EGFR1b: 5'- TCGCCGCCAACGCCACAAC-3' (forward) (SEQ ID NO: 1) and 5'- ACACGCCCTTACCTTTCTTTTCCTCCAG-3' (reverse) (SEQ ID NO: 2); EGFR2a: 5'- CCGCGAGTTTCCCTCGCATTTCT-3' (forward) (SEQ ID NO: 3)

and 5'- CCTTCCCCCTTTCCCTTCTTTTGTTTTAC-3' (reverse) (SEQ ID NO: 4); EGFR2b: 5'- TCCCATTTCCTTTCTCTAGTTTTGTTTTTC-3' (forward) (SEQ ID NO: 5) and 5'- GTCCACCCCATCCCCACTGTTCTTCTC-3' (reverse) (SEQ ID NO: 6); EGFR3: 5'- TTCAGCAAACCCATTCTTCT-3' (forward) (SEQ ID NO: 7) and 5' GCTTCCTGCACACCTGGGCTGAG-3' (reverse) (SEQ ID NO: 8). The PCR program was set with an initial melting step at 94°C for 3min, then 35 cycles of (94°C for 20 sec, 59°C for 30 sec, and 72°C for 30 sec). The PCR products were then analyzed on agarose gel by electrophoresis. PCR products were taken out after 32, 36 and 40 cycles to ensure that the reaction was in the linear range. 35 cycles was sufficient to produce amplicons in the linear range, and was used to establish optimal PCR conditions for the EGFR primers.

[0087] Endogenous YB-1 was subjected to ChIP using a chicken anti-human YB-1 polyclonal antibody, using a protocol adapted from the Chromatin Immunoprecipitation Assay Kit (Upstate). In brief, 7.5×10^6 cells per plate were treated with 1% formaldehyde in growth media then harvested, pelleted and washed with PBS. Pellets were resuspended in lysis buffer (1% SDS, 10mM EDTA, 50mM Tris, (pH 8.1)) and sheared on ice by sonication (25% power, 10 cycles of 20 seconds on, 30 seconds off). Chromatin solution was diluted 5-fold in immunoprecipitation buffer (0.01% SDS, 1% Triton X-100, 1.2mM EDTA, 16.7mM Tris (pH 8.1), 500mM NaCl) and precleared with PrecipHen beads (Aves Labs #P-1010) in lysis buffer (1:3). The solution was then incubated with anti-YB-1, chicken IgY, or pre-immune chicken IgY overnight. Complexes were incubated with 100ul of PrecipHen beads in lysis buffer (1:3) for 1.5 hrs. Immunoprecipitates were washed once with low salt buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris (pH 8.0), 150mM NaCl), once with high salt buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris (pH 8.0), 500mM NaCl), once with LiCl buffer (0.25M LiCl, 1% IGEPAL, 1% deoxycholate, 1mM EDTA, 10mM Tris (pH 8.0)), and twice with Tris-EDTA buffer (10mM Tris pH 8.0, 1mM EDTA). Protein:DNA complexes were eluted twice with elution buffer (1% SDS, 0.1M NaHCO₃) and brief

vortex. Eluates and input controls were treated with 5M NaCl at 65°C overnight followed by proteinase K at 45°C for 1 hr. DNA was purified by phenol/chloroform extraction and alcohol precipitation. PCR was performed with primers to EGFR as described above, or to Her-2. Her-2 primers used were: 5'-AGGGGCTCCAAATAGAATGT (forward) (SEQ ID NO: 9), 5'-AATTTGGGAGGAGACAGTCA (reverse) (SEQ ID NO: 10). The Her-2 primers produced a 464 bp product that spanned -978 to -514 of the HER2 promoter. The DNA was amplified using the following PCR conditions: 95°C for 2min, 40 cycles of 95°C for 20sec, 57°C for 30sec, 72°C for 30sec.

Small interfering RNA

[0088] Small interfering RNA targeting YB-1 was expressed in pSuperDuper as previously described (*EN-NIA et al. 2004 J Biol Chem* 280:7702-7711). The targeting vector was used to silence YB-1 in MCF-7(Flag:YB-1) cells. MCF-7(Flag:YB-1) cells were plated in a 6-well dish and transfected with 0.8 ug of targeting vector plasmid DNA. The plasmid DNA was added to the transfection reagent (Lipofectamine 2000, InVitrogen) at a ratio of 1:12.5 (siYB-1 #1) or 1:25 (siYB-1#2), 96 hrs later the cells were lysed in ELB buffer and evaluated for changes in YB-1, EGFR and Her-2 by Western immunoblotting.

Electrophoretic mobility shift and supershift assays

[0089] Electrophoretic mobility shift assays (EMSA) were performed using the LightShift Chemiluminescent EMSA Kit (Pierce product # 20148), following manufacturer's instructions, with some modifications. Briefly, nuclear protein extracts from MDA-MB-468 cells were obtained with the NE-PER Nuclear and Cytoplasmic Extraction Reagent Kit (Pierce, product # 78833), following manufacturer's instructions. 4 ug of nuclear extract protein was used per reaction. Oligonucleotide probes corresponding to EGFR promoter sequences (SEQ ID NO: 13 and SEQ ID NO: 14) were synthesized and labelled at the 5' end with biotin. For YB-1 antibody supershift assays, 1 or 2 ug of YB-1 chicken polyclonal antibody was combined with the labelled

probe and nuclear extract (no unlabelled probe). For the peptide inhibition assays, nuclear extracts were pretreated with 0 (DMSO control), 12,5, 25 or 50 uM peptide corresponding to SEQ ID NO: 11, followed by incubation with labelled EGFR peptide. Samples were electrophoretically separated on 6%
5 non-denaturing polyacrylamide gel, transferred to nylon membrane and crosslinked, and visualized using chemiluminescence.

Peptide synthesis

[0090] Biotin conjugated 25-mer peptides (AP-CSD) comprising the
10 *Antennapedia* translocation domain and an additional 9 amino acids were synthesized using methods known in the art (Sigma, St. Louis MO) (RQIKIWFQNRRMKWKKPRKYLRSVG) (SEQ ID NO: 11).

Example 1

[0091] YB-1 binds directly to the EGFR and Her-2 promoters

Nine putative YB-1 responsive elements were identified in the first 2 kb of the EGFR promoter (Figure 2A). DNA:protein complexes were isolated from MCF-7/Flag:YB-1 and MCF- 7/Flag:YB-1(A102) expressing cells. The input DNA was evaluated in several ways prior to chromatin immunoprecipitation
20 (ChIP). First, a sample of the sheared DNA was evaluated from each sample. The degree of shearing and the amount of input DNA appeared equal. Genomic DNA was amplified with each of the primer sets to produce a single gene product of the expected size. ChIP was then performed using an antibody to Flag. The anti-Flag:YB-1 antibody was able to precipitate DNA
25 that was amplified with each of the EGFR primer sets, suggesting that YB-1 bound directly to the EGFR promoter region (Figure 2B, lanes 1-4). DNA pulled down from MCF-7/Flag:YB-1(A102) could be amplified by the EGFR 2b and EGFR3 primer sets (Figure 2B, lanes 7-8); however, there was no amplification when EGFR 1b and EGFR2a primer sets were used (Figure 2B,
30 lanes 5-6). The absence of PCR product from these sites with MCF-7/Flag:YB-1(A102) samples was not due to primer inefficiency as the EGFR 2a primers were able to produce PCR products from the MCF-7/Flag:YB-

1(A102) genomic DNA (Figure 2B, lanes 16-18) . To ensure that the input DNA was equal prior to performing ChIP, aliquots of the input DNA from each sample was titrated (1, 3, 5 ul). The input DNA was amplified with primers to EGFR 2a and the amount of PCR products from MCF-7/Flag:YB-1 and MCF-7/Flag:YB-1(A102) appeared the same (Figure 2B, lanes 13-18). Given these data, we were confident that our ChIP starting material was unbiased. The loss of YB-1(S102) thus disrupted binding to both the EGFR 1b and EGFR 2a sites located in the proximal promoter. These data indicated that YB-1(A102) does not optimally bind to the first 1kb of the EGFR promoter, which could explain why loss of S102 prevents the induction of EGFR.

[0092] Following this series of experiments, ChIP was performed on the MDA-MB-231 breast cancer cells to confirm that endogenous YB-1 bound to the EGFR promoter. The primers EGFR1b, EGFR2a and EGFR2b amplified products of the expected size; however EGFR3 did not (Figure 2C, lanes 2-5). Amplification was not due to non-specific binding to the IgY given that the control reactions produced little or no product (Figure 2C, lanes 6-9). The sheared input DNA (Figure 2D, lanes 2-6) and the no template DNA reactions (Figure 2D, lanes 6-9) served as positive and negative controls for ChIP. The primers were also used to amplify genomic DNA (Figure 2D, lanes 10-13). We concluded that Flag:YB-1 and endogenous YB-1 bound to the EGFR promoter and that loss of the S102 site on YB-1 destabilized the interaction.

[0093] Similarly, we investigated whether disrupting YB-1 at S102 has an impact on the binding of this transcription factor to Her-2. There are four potential YB-1 responsive elements (YRE's) on the Her-2 promoter (Figure 6A). Primers were designed to capture three out of four binding sites excluding the one that was juxtaposed to the AP-2 and Ets sites. Flag:YB-1 bound to Her-2 while the Flag:YB-1(A102) mutant did not (Figure 6B). We further questioned whether endogenous YB-1 also bound to Her-2 and to interrogate this possibility we used the MDA-MB- 453 breast cancer cells because they express very high levels of Her-2 in the absence of gene

amplification. Endogenous YB-1:DNA complexes were pulled down and the precipitated DNA was amplified for Her-2. In this breast cancer cell line, YB-1 specifically bound to the Her-2 promoter (Figure 6C, lane 2), further supported by the absence of binding to the IgY (Figure 6C, lane 3). Input sheared DNA served as a positive control (Figure 6C, lane 4).

Example 2

EGFR and HER2 are regulated by YB-1

[0094] Flag:YB-1 was stably transfected into MCF-7 cells, to investigate a possible mechanistic link between YB-1 and expression of EGFR and HER2. Comparisons were made to cells expressing mutant YB-1(A102) that had the S102 site disrupted by site-directed mutagenesis. The level of the transgene expression was approximately the same as endogenous YB-1. Surprisingly, Her-2 and EGFR proteins were induced as a result of YB-1 over-expression (Figure 3A). However, mutating YB-1 on its DNA binding domain from S102 to A102 prevented the induction of those proteins (Figure 3A). Additionally, the expression of YB-1 caused an increase in EGFR and Her-2 mRNA whereas the mutant did not (Figure 3B, C). Cells expressing Flag:YB-1 were also more responsive to EGF because there was more signaling through the MAP kinase pathway (Figure 3D, middle lane) as compared to the cells expressing either the YB-1(A102) (Figure 3D, right lane) or empty vector (Figure 3D, left lane).

Example 3

YB-1 positively correlates with EGFR and Her-2 in primary breast tumours

[0095] Tumour tissue microarrays representing 389 primary breast tumours were immunostained for YB-1, EGFR, Her-2, ER and Ki67. YB-1 protein was coordinately expressed with Her-2 and EGFR as well as the proliferation marker Ki67 (Table 4). YB-1 expression was also strongly associated with ER negative breast cancers. Nodal status and tumor size did not relate to high levels of YB-1. YB-1 was moderately to highly expressed in

43% (167/389) of the cases, which showed decreased patient survival based on Breslow ($p=0.0065$) and log rank ($p=0.01$) analyses, respectively (Figure 1). The Breslow test is predictive of early deaths. These data indicate that YB-1 expression is associated with early death due to breast cancer. In a multivariate analysis, YB-1 had independent prognostic significance ($p=0.019$) (Table 5); the Cox regression model included Her-2 ($p=0.018$), lymph node status ($p<0.001$) and tumor size ($p=0.022$), which are also known to be independently associated with poor survival. YB-1 had a comparable impact on the relative risk of dying from breast cancer to expression of either HER2 or tumor size. The relative risk ratios for YB-1, HER2, lymph node status and tumor size were 1.792, 1.994, 2.436 and 1.788, respectively.

Table 4. YB-1 correlation with primary breast tumor markers

Marker	Fisher's Exact	Patient Number
EGFR	0.000*	337
HER2	0.008*	319
ER	0.000*	304
Ki67	0.002*	304
Nodal Status	0.909	350
Size (>2 cm).	0.57	321

*Correlations were considered significant if the Fisher's Exact test were $p<0.05$

Table 5. Multivariate analysis of YB-1 in comparison to other prognostic markers for breast cancer.

Variable	Significance	Relative Risk	95% CI
YB-1	0.019	1.792	1.103-2.913
HER2	0.018	1.994	1.126-3.561
Lymph node status	<0.001	2.436	1.484-3.997
Tumor size	0.022	1.788	1.089-2.935

Example 4

YB-1 siRNA results in decrease of EGFR, HER2 protein expression

[0096] The expression of YB-1 was silenced using small interfering RNA's. MCF-7(Flag:YB-1) cells were transfected with pSuperDuper vector expressing small interfering RNA targeting YB-1, and proteins were harvested 96 hours later. The targeting vector suppressed the expression of Flag:YB-1 and endogenous YB-1 (Figure 4A, lanes 2&3) compared to the empty vector (Figure 4A, lane 1). Loss of YB-1 expression correlated with a decrease in EGFR protein expression (Figure 4A, second panel). Two different concentrations of the targeting vector were used (siYB-1#1 and siYB-1#2) and both resulted in suppression of EGFR. Likewise, knocking down YB-1 inhibited the expression of Her-2 (Figure 4B). Similarly, knocking down YB-1 with a pSuper vector targeting different portion of YB-1 suppressed the expression of Her-2 in MDA-MB-453 cells (not shown). YB-1 was necessary for the expression of these receptors.

Example 5

YB-1 inhibition suppresses growth of Herceptin resistant and EGFR-expressing cells

[0097] Herceptin resistant cells (MDA-MB-453; see *Kucab et al 2005, Breast Cancer Res. 7(5):R796-807; Menendez et al 2005, J Natl Cancer Inst. 2;97(21):1611-5*) or EGFR over-expressing (SUM 149; see *Kleer et al 2002, Oncogene 9;21(20):3172-80; Kleer et al 2004, Neoplasia 6(2):179-85; Van den Eynden GG, Van Laere SJ, Van der Auwera I, Merajver SD, Van Marck EA, van Dam P, Vermeulen et al 2005, Breast Cancer Res Treat. 22;:1-10; Lev et al 2004, Br J Cancer. 16;91(4):795-802*) were exposed AP-CSD (SEQ ID NO: 11) peptide conjugated to a biotin tracer at a concentration of 0 (DMSO control), 12.5, 25 or 50 μ M for 72 hours, and allowed to grow. Growth of cells under these conditions was assessed by MTT assay. MDA-MB-453 cells (Figure 5A) demonstrate significant growth inhibition at peptide

concentrations of 25 μ M and greater, knocking back cell survival to less than 10% of control cells. SUM 149 cells (Figure 5B) also demonstrated significant growth inhibition at peptide concentrations of 25 μ M, with cell survival decreased to ~60% of control, and less than 5% of control at 50 μ M.

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Example 6

YB-1:DNA binding to the EGFR promoter is inhibited with the cell permeable peptide

10 **[0098]** Nuclear extracts were taken from a breast cancer cell line that expresses high levels of EGFR, the MDA-MB-468 line, and assayed for EGFR-promoter binding and the presence of YB-1. YB-1 binds directly to the EGFR promoter using a gel shift assay (Figure 7A). Lane 1 shows the unbound, biotin-labelled EGFR probe, lane 2 shows the shift of the EGFR
15 probe when bound by protein in the nuclear extract. Lane 3 demonstrates the specificity of the binding to the EGFR probe by pre-incubation of the nuclear extract with unlabelled probe. YB-1 specific antisera (lanes 4 and 5) was combined with bound, labeled probe, demonstrating the 'supershift' effect of the bound YB-1 antibody.

20

25 **[0099]** AP-CSD (SEQ ID NO: 11) peptide conjugated to biotin was used to inhibit binding of YB-1 to the labelled EGFR probe (Figure 7B). Lane 1 shows the labeled EGFR probe bound by protein from the nuclear extract. For lanes 2, 3 and 4, the nuclear extract was pre-incubated with 12.5, 25 or 50 μ M biotin labeled peptide before incubating with the labeled EGFR probe. Binding of YB-1 to the EGFR promoter sequence was inhibited by the AP-CSD peptide.

25

Example 7

Efficacy of peptide in other cell lines

30 **[00100]** PC3 prostate adenocarcinoma cells represent an aggressive, incurable form of prostate cancer. When PC3 cells were exposed biotin-labelled AP-CSB peptide (SEQ ID NO: 11) at a concentration of 0 (DMSO

control), 12.5, 25 or 50 μ M for 72 hours and allowed to grow. Growth of cells under these conditions were assessed by MTT assay (Figure 8). Growth of PC3 cells was suppressed in a dose-dependent manner by the peptide.

5

Example 8

YB-1 phosphorylation inhibiting peptide does not significantly inhibit normal breast epithelial cell growth

[00101] The two protocols set out in this example together provided evidence that YB-1 inhibitory peptides of the invention preferentially kill breast cancer cells.

10

[00102] Normal mammary EpCAM+ breast epithelial cells were taken from two women who had undergone a reduction mammoplasty. The cells were isolated, plated at a density of 1000 cells/well, treated with either a control (RQIKIWFQNRRMKWKKRALKYGVRP) or YB-1 inhibitory peptides (6 or 25 μ M RQIKIWFQNRRMKWKKPRKYLRSVG Seq ID no: 11) for 72 hrs and then assessed for viability using the MTS assay. Both peptides were synthesized by Sigma to >95% Purity, 50mg according to Mass Spectral and HPLC Analyses and were reconstituted in acetic acid. The samples were evaluated in replicates of six. The results illustrated in Figure 9 confirm that the YB-1 inhibitory peptide has essentially no toxicity on normal mammary EpCAM+ cells. The results of a colony forming assays for the normal breast epithelial cells (data not shown) also confirmed that the YB-1 peptide has essentially no adverse effect on the ability of the normal cells to form colonies.

15

20

25

[00103] The peptides were tested as above against pre-neoplastic 184htrt cells (shown to lack YB-1 expression, data not illustrated herein, a cell line previously described in *Oh et al 2002, Neoplasia 4(3) 204-217*) and compared to the breast cancer SUM149 cells, to test for specificity. The YB-1 inhibitory peptide (SEQ ID No: 11) killed >90% of the SUM149 cells at concentrations above 12.5 μ M, whereas there was only a modest deleterious effect on the 184htrt cells, particularly at concentrations below 12.5 μ M,

30

demonstrating that the peptide has modest off target effects, as illustrated in Figure 10.

REFERENCES

5 **[00104]** The following list of references is a specific listing that goes beyond the references cited above, to provide a bibliographic summary of art that relates in large part to protein transduction domains. These references are hereby incorporated by reference, as are the other documents cited herein:

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CONCLUSION

20 **[00168]** Although various embodiments of the invention are disclosed herein, many adaptations and modifications may be made within the scope of the invention in accordance with the common general knowledge of those skilled in this art. Such modifications include the substitution of known equivalents for any aspect of the invention in order to achieve the same result

25 in substantially the same way. Numeric ranges are inclusive of the numbers defining the range. The word "comprising" is used herein as an open-ended term, substantially equivalent to the phrase "including, but not limited to", and the word "comprises" has a corresponding meaning. As used herein, the singular forms "a", "an" and "the" include plural referents unless the context

30 clearly dictates otherwise. Thus, for example, reference to "a thing" includes more than one such thing. Citation of references herein is not an admission that such references are prior art to the present invention. Any priority

document(s) and all publications, including but not limited to patents and patent applications, cited in this specification are incorporated herein by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein and as though fully set forth
5 herein. The invention includes all embodiments and variations substantially as hereinbefore described and with reference to the examples and drawings.

CLAIMS

1. A cell permeable Y-box binding protein-1 (YB-1) phosphorylation inhibitor comprising a peptidic sequence that is a YB-1 selective protein kinase B (Akt) phosphorylation recognition site.

5

2. The YB-1 phosphorylation inhibitor of claim 1, wherein the Akt phosphorylation site comprises the sequence RXXXRS, wherein X is any amino acid or amino acid analogue.

10

3. The YB-1 phosphorylation inhibitor of claim 1, comprising a contiguous sequence of at least nine residues of the formula:

$$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12}$$

wherein:

X_1 is Z or N or D or H or S or X

15

X_2 is Z or N or D or H or S or X

X_3 is Z or P

X_4 is R

X_5 is K or R or E or Q or X

X_6 is Y or F or W or X

20

X_7 is L or M or I or V or F or X

X_8 is R

X_9 is S

X_{10} is Z or V or I or L or M or X

X_{11} is Z or G or X

25

X_{12} is Z or D or N or E or X

in which

X = any amino acid or amino acid analogue,

Z = not present.

30

4. The YB-1 phosphorylation inhibitor of claim 3, wherein:

X_1 is Z or N or D

X_2 is Z or N or D

X_3 is Z or P
 X_4 is R
 X_5 is K or R
 X_6 is Y or F or W
 X_7 is L or M or I
 X_8 is R
 X_9 is S
 X_{10} is Z or V or I
 X_{11} is Z or G
 X_{12} is Z or D or N or E.

5

10

5. The YB-1 phosphorylation inhibitor of any one of claims 1 to 4, further comprising a protein transduction domain.

15

6. The YB-1 phosphorylation inhibitor of claim 5, wherein the protein transduction domain is an *Antennapedia* transduction domain.

20

7. The YB-1 phosphorylation inhibitor of claim 6, wherein the *Antennapedia* transduction domain has the sequence RQIKIWFQNRRMKWKK.

25

8. The YB-1 phosphorylation inhibitor of any one of claims 1 through 7, wherein the YB-1 selective Akt phosphorylation site has the sequence PRKYLRSVG.

30

9. The YB-1 phosphorylation inhibitor of claim 1, consisting essentially of a peptide having the sequence RQIKIWFQNRRMKWKKPRKYLRSVG.

10. A composition comprising the YB-1 phosphorylation inhibitor of any one of claims 1 to 9 and a pharmaceutically acceptable carrier.

11. Use of the YB-1 phosphorylation inhibitor of any one of claims 1 to 9, to formulate a medicament.
- 5 12. Use of the YB-1 phosphorylation inhibitor of any one of claims 1 to 9, to formulate a medicament for treating a YB-1 mediated disease.
13. Use of the YB-1 phosphorylation inhibitor of any one of claims 1 to 9, for treating a YB-1 mediated disease.
- 10 14. The use of the YB-1 phosphorylation inhibitor according to claim 12 or 13, wherein the YB-1 mediated disease is selected from the group consisting of cancer, heart disease, kidney disease, diabetes, autoimmune disease, cerebral palsy and infection.
- 15 15. The use of the YB-1 phosphorylation inhibitor according to claim 12 or 13, wherein the YB-1 mediated disease is a cancer.
- 20 16. The use of the YB-1 phosphorylation inhibitor according to claim 15, wherein the cancer is selected from the group consisting of melanoma, adenocarcinoma, hepatoma, fibrosarcoma, colon, prostate and breast cancer.
- 25 17. The use of the YB-1 phosphorylation inhibitor according to claim 15, wherein the cancer is a breast cancer.
- 30 18. The use of the YB-1 phosphorylation inhibitor according to claim 17, wherein the breast cancer is a trastuzumab resistant breast cancer.
19. The use of the YB-1 phosphorylation inhibitor according to claim 15, wherein the cancer is a prostate cancer.

20. The use of the YB-1 phosphorylation inhibitor according to claim 19, wherein the prostate cancer is an androgen independent prostate cancer.
- 5 21. A method of treating a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the YB-1 phosphorylation inhibitor of any one of claims 1 to 9.
- 10 22. The method of claim 21, wherein the subject is suffering from a YB-1 mediated disease.
- 15 23. The method of claim 21, wherein the subject is suffering from a disease selected from the group consisting of cancer, heart disease, kidney disease, diabetes, autoimmune disease, cerebral palsy and infection.
- 20 24. The method of claim 23, wherein the disease is a cancer.
- 25 25. The method of claim 24, wherein the cancer is selected from the group consisting of melanoma, adenocarcinoma, hepatoma, fibrosarcoma, colon, prostate and breast cancer.
26. The method of claim 25, wherein the cancer is a breast cancer.
27. The method of claim 26, wherein the breast cancer is a trastuzumab resistant breast cancer.
28. The method of claim 25, wherein the cancer is a prostate cancer.
- 30 29. The method of claim 28, wherein the prostate cancer is an androgen-independent prostate cancer.

30. The method of any one of claims 21 to 29, wherein the subject is a human.
- 5 31. A method of inhibiting the phosphorylation of YB-1 comprising contacting a cell with the YB-1 phosphorylation inhibitor of any one of claims 1 to 9.
32. The method of claim 31, wherein the cell is a human cancer cell.
- 10 33. The method of claim 32, wherein the cancer cell is a breast cancer cell.
34. The method of claim 33, wherein the breast cancer cell is a trastuzumab resistant breast cancer cell.
- 15 35. The method of claim 32, wherein the cancer cell is a prostate cancer cell.
36. The method of claim 35, wherein the prostate cancer is an androgen-independent prostate cancer cell.
- 20 37. A recombinant nucleic acid encoding the YB-1 phosphorylation inhibitor of any one of claims 1 to 9, wherein the YB-1 phosphorylation inhibitor consists of a polypeptide.
- 25 38. A cell permeable peptidic compound comprising an Akt phosphorylation recognition site comprising the peptide sequence PRKYLRSVG.
- 30 39. A cell permeable peptidic compound comprising an Akt phosphorylation recognition site comprising the peptide sequence:

$$X_1 X_2 X_3 X_4 X_5 X_6 X_7 X_8 X_9 X_{10} X_{11} X_{12}$$
wherein:

X₁ is Z or N or D or H or S or X

X₂ is Z or N or D or H or S or X

X₃ is Z or P

X₄ is R

5

X₅ is K or R or E or Q or X

X₆ is Y or F or W or X

X₇ is L or M or I or V or F or X

X₈ is R

X₉ is S

10

X₁₀ is Z or V or I or L or M or X

X₁₁ is Z or G or X

X₁₂ is Z or D or N or E or X

in which

X = any amino acid or amino acid analogue,

15

Z = not present.

40. The compound of claim 39, wherein:

X₁ is Z or N or D

X₂ is Z or N or D

20

X₃ is Z or P

X₄ is R

X₅ is K or R

X₆ is Y or F or W

X₇ is L or M or I

25

X₈ is R

X₉ is S

X₁₀ is Z or V or I

X₁₁ is Z or G

X₁₂ is Z or D or N or E.

30

41. The compound of any one of claims 38 to 40, further comprising a protein transduction domain.

Figure 1

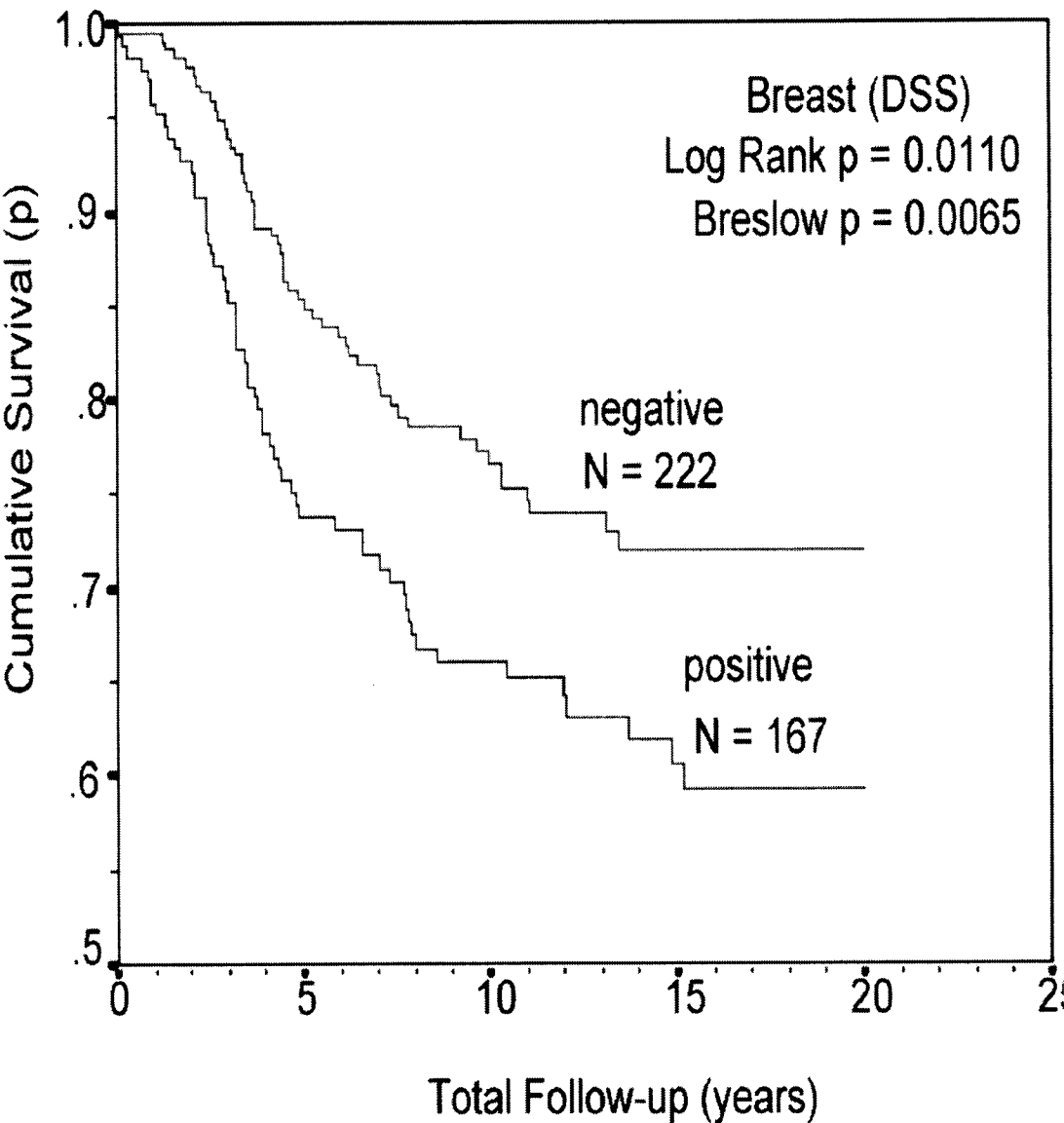


Figure 2

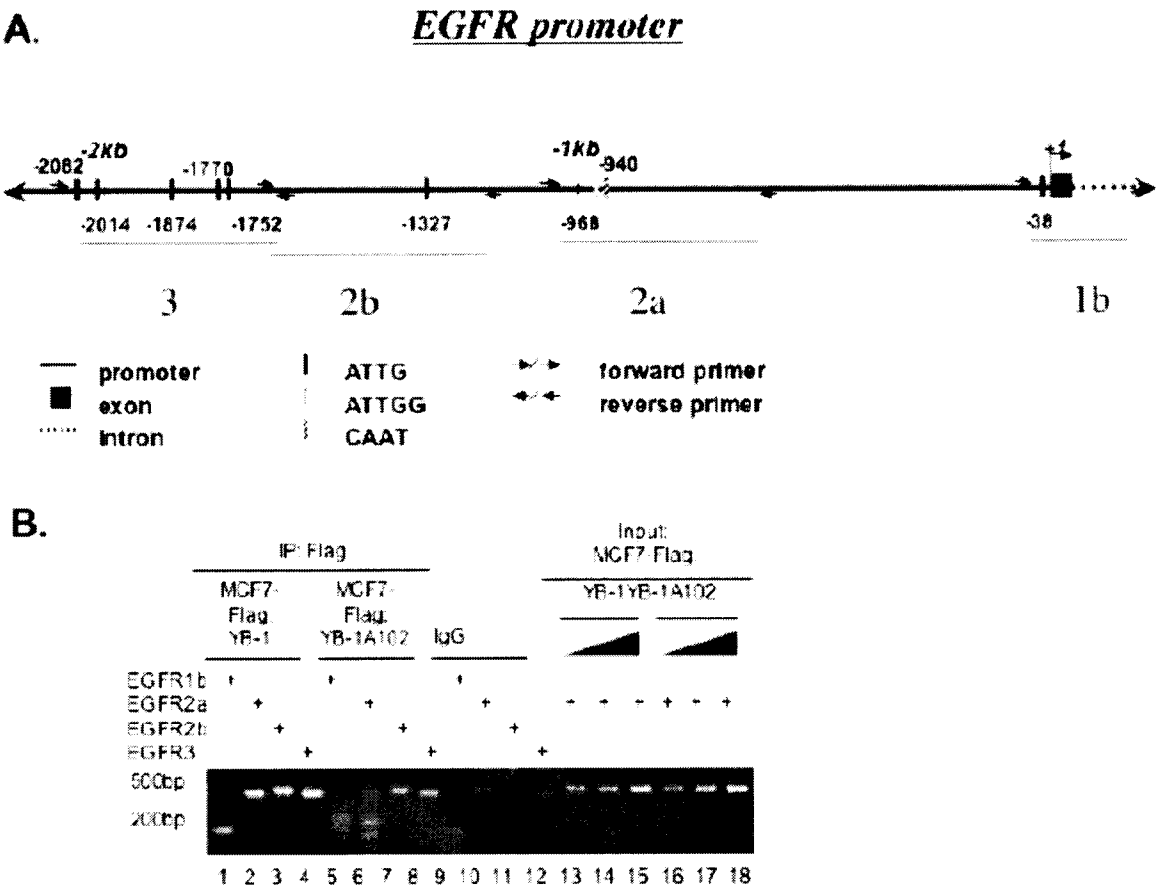
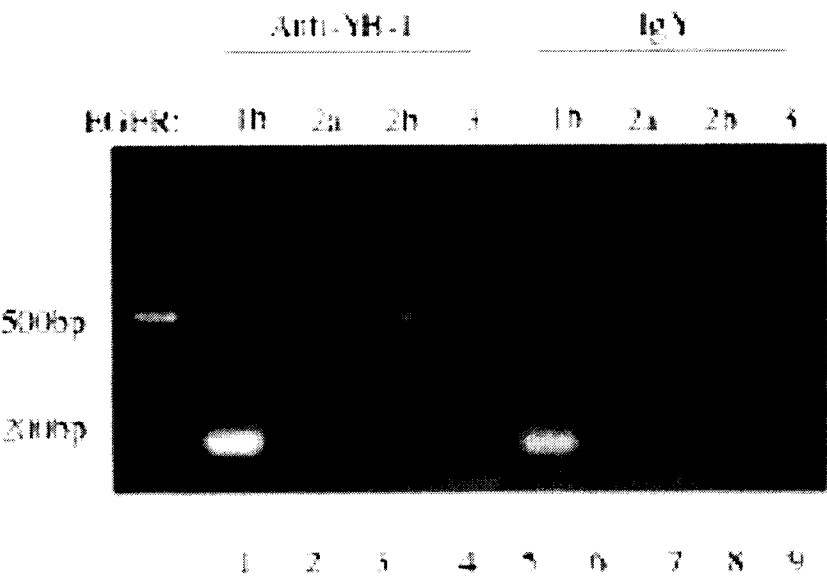


Figure 2 (continued)

C.



D.

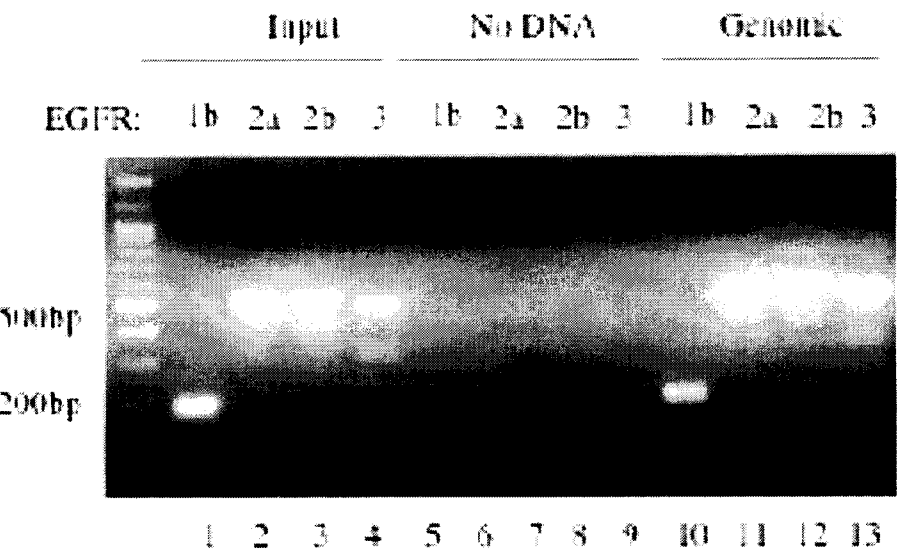


Figure 3

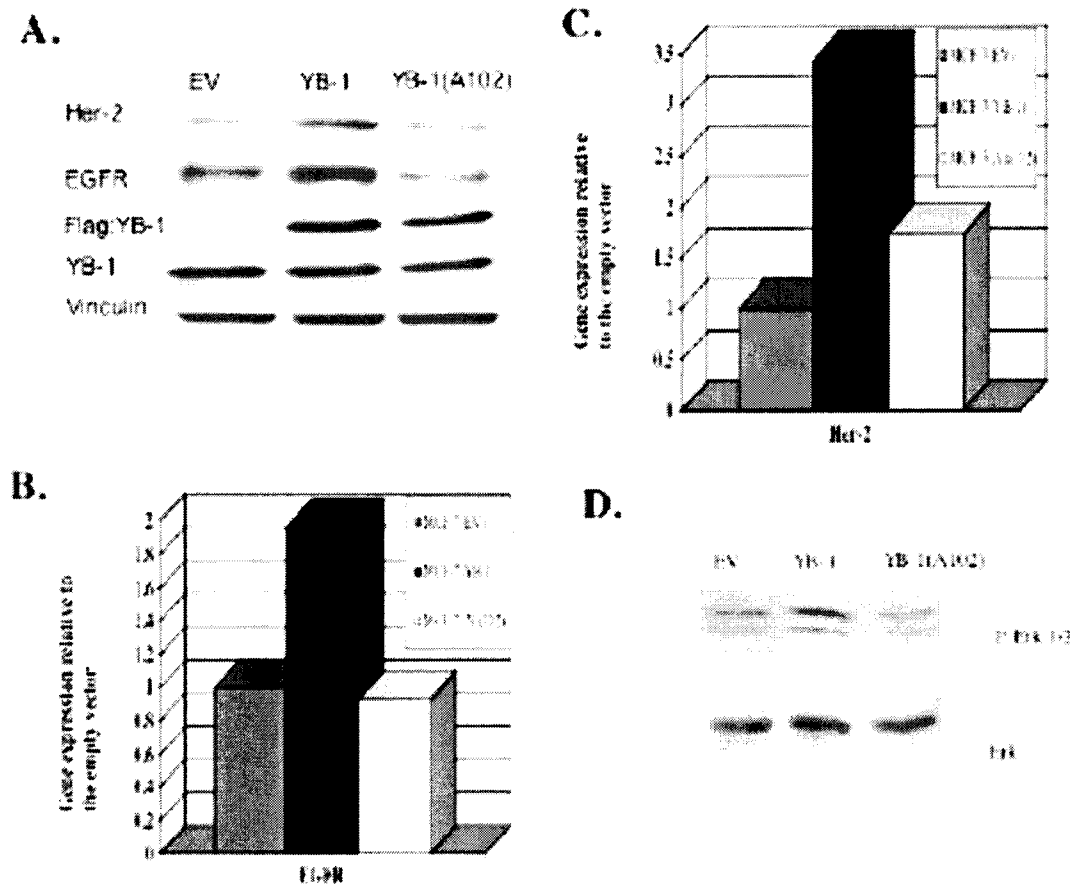


Figure 4

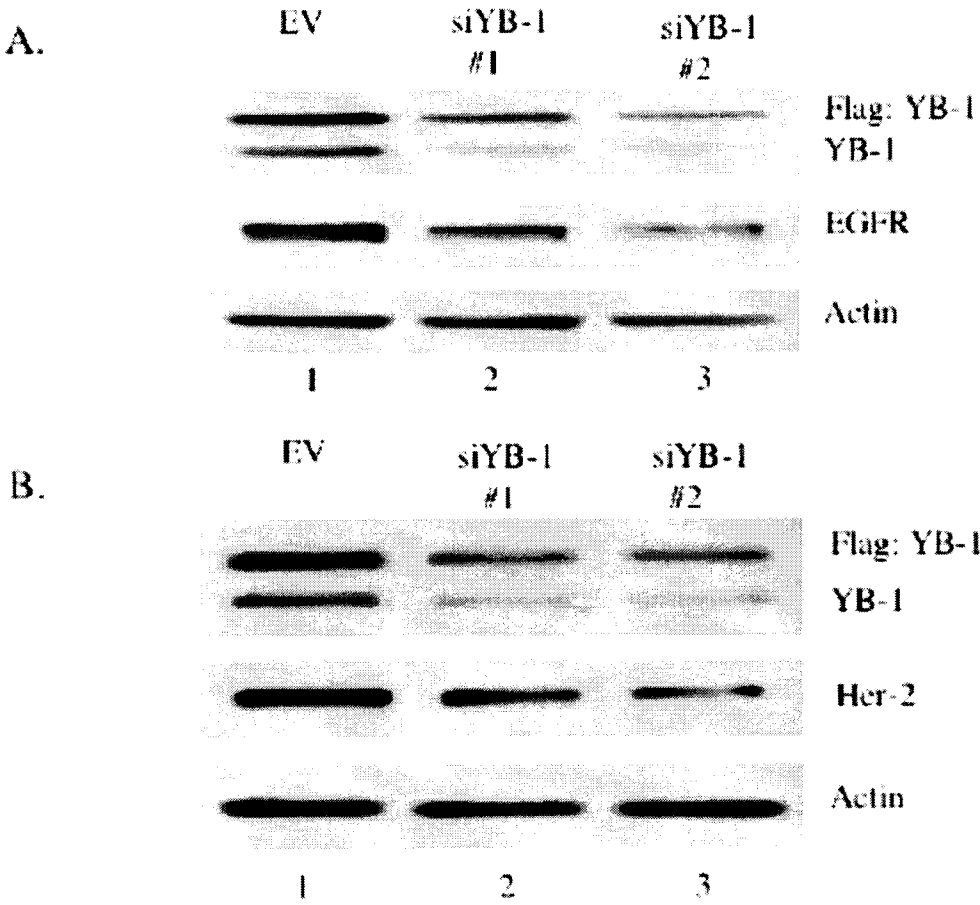


Figure 5

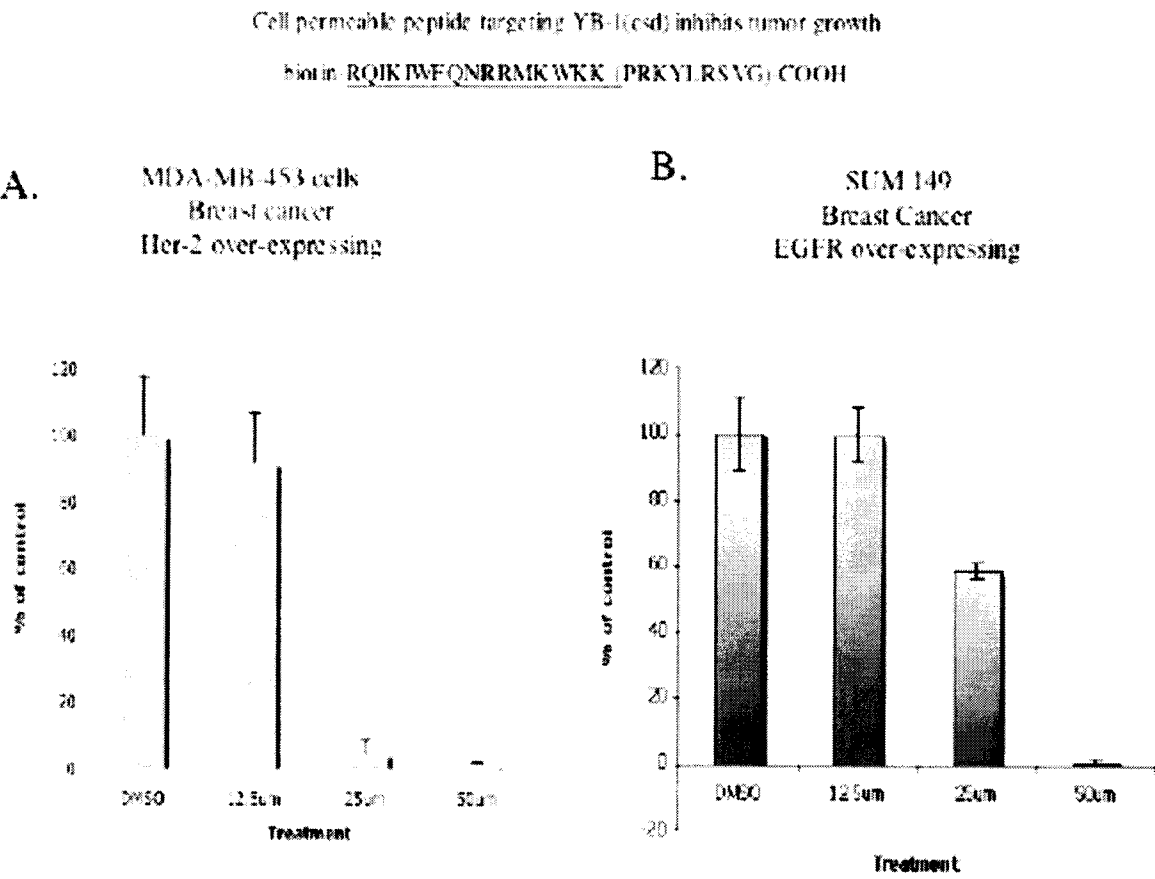
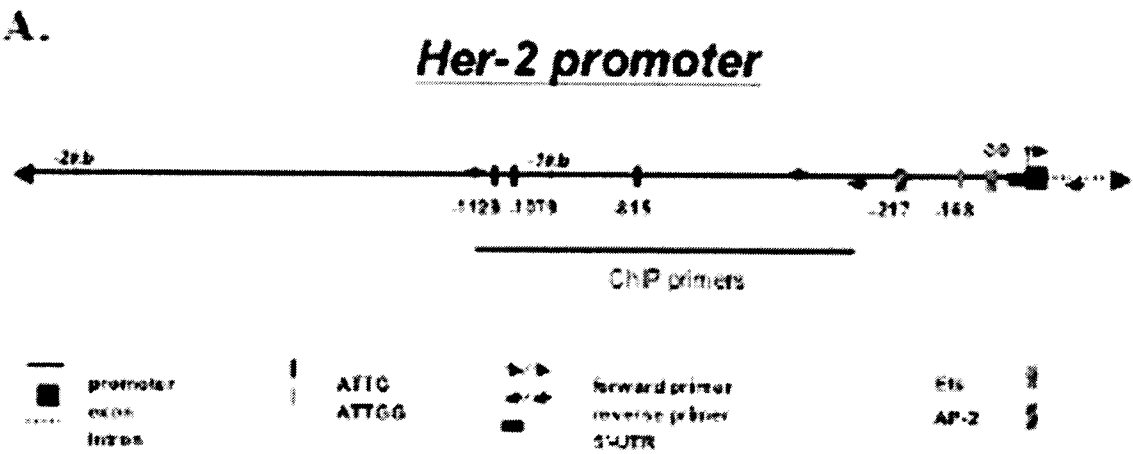
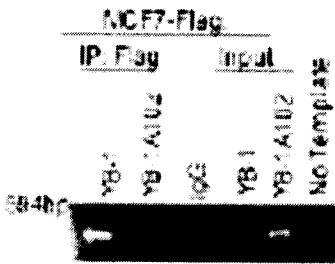


Figure 6



B.



C.

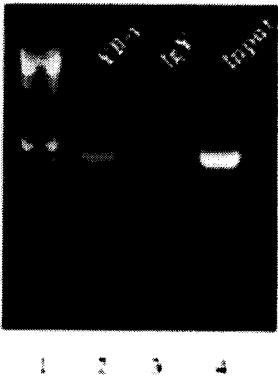


Figure 7

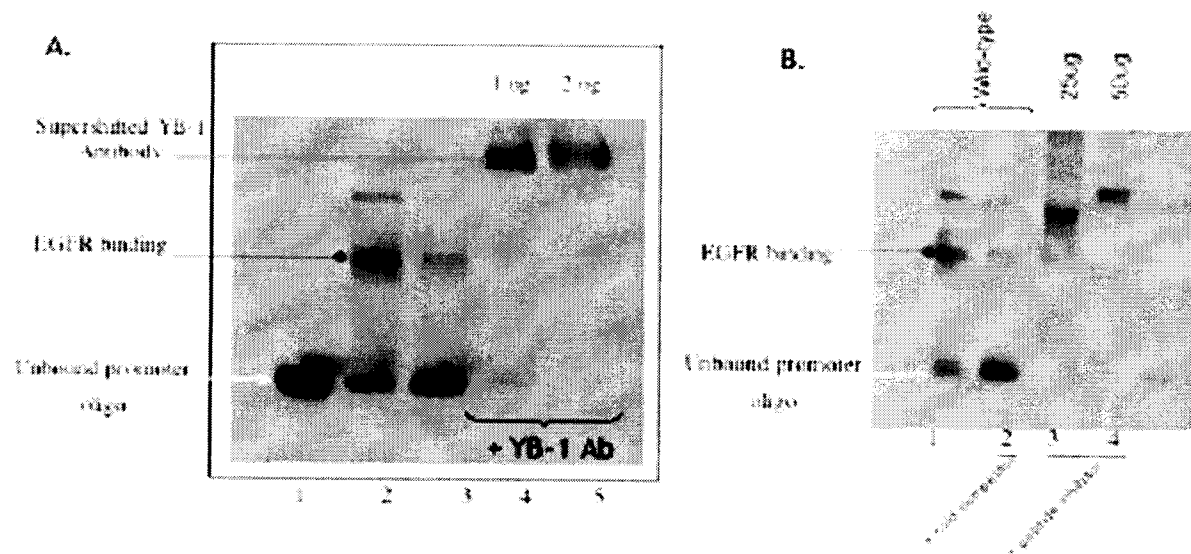


Figure 8

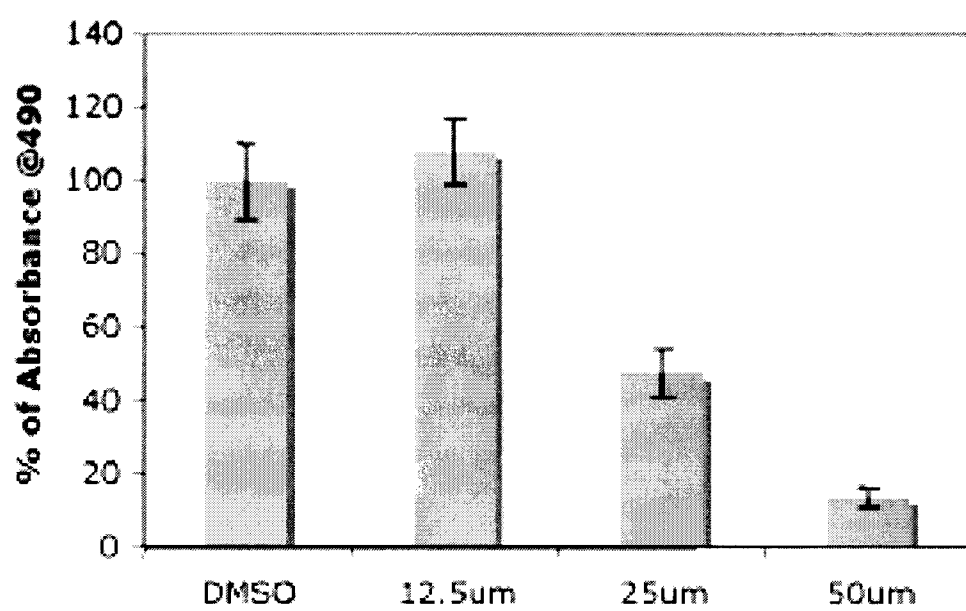


Fig 9

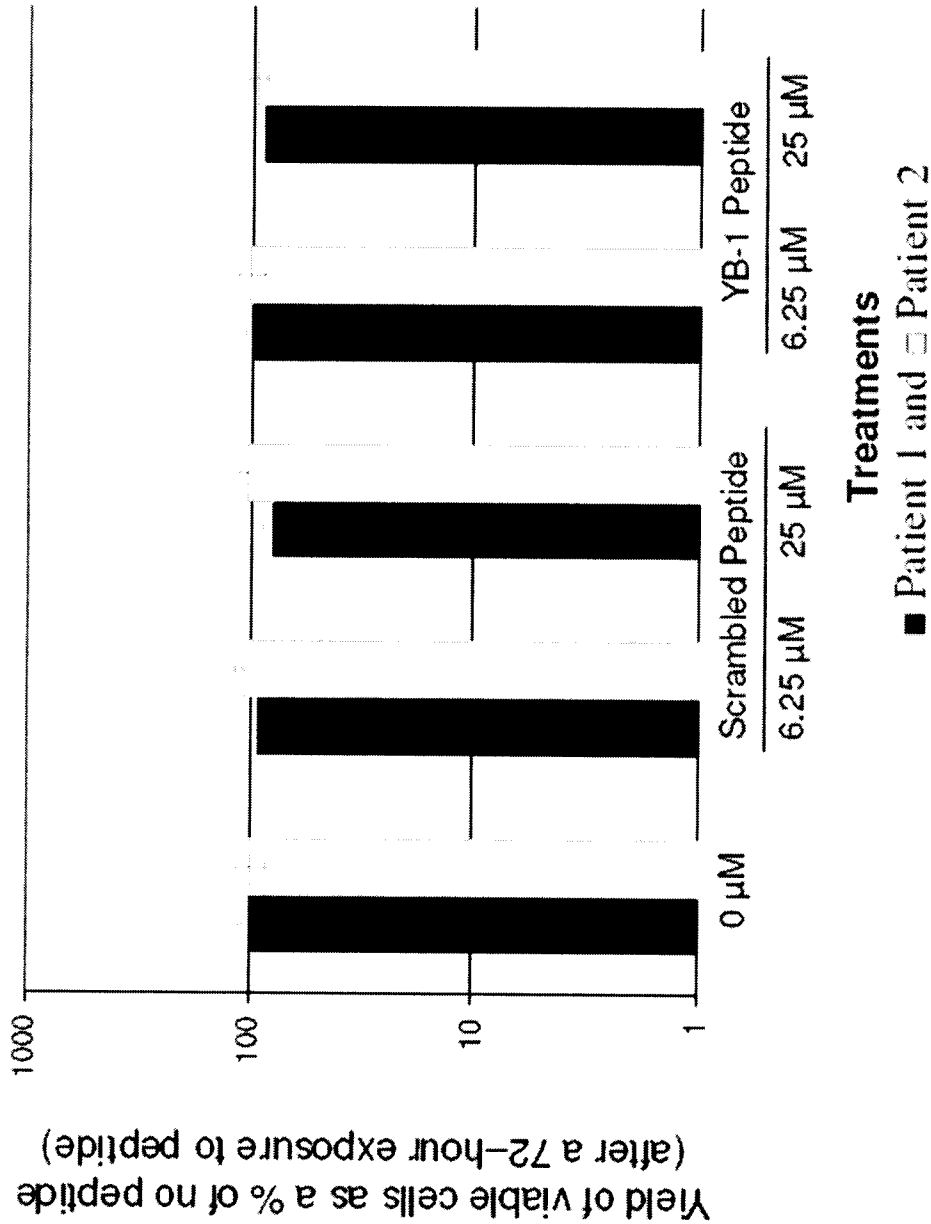
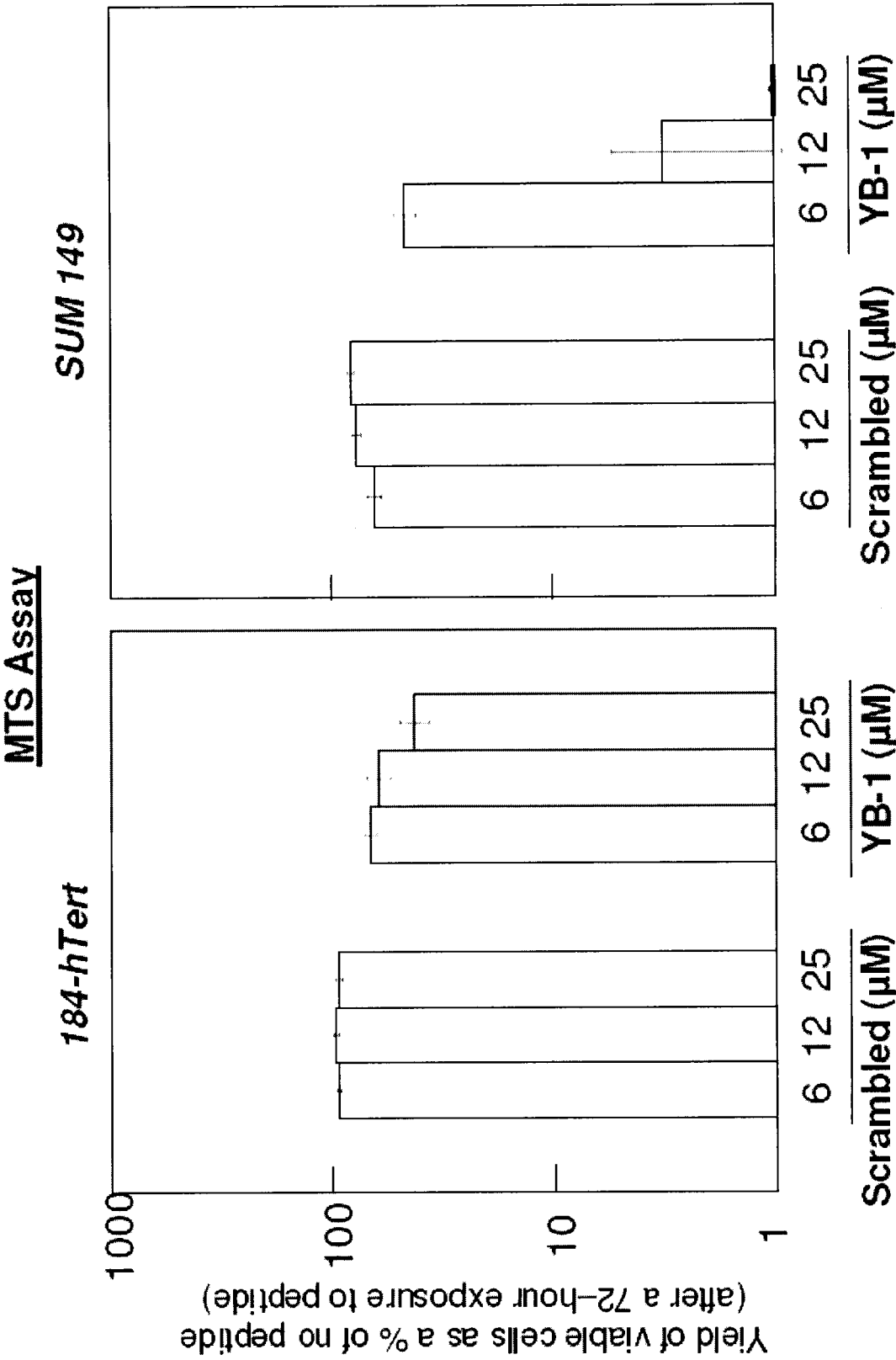


Fig. 10



INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2007/000526

A. CLASSIFICATION OF SUBJECT MATTER

IPC: *C12N 15/12* (2006.01) , *A61K 38/08* (2006.01) , *A61K 38/17* (2006.01) , *A61P 35/00* (2006.01) ,
C07K 14/47 (2006.01) , *C07K 7/06* (2006.01), *C07K 7/08* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 15/12 (2006.01) , *A61K 38/08* (2006.01) , *A61K 38/17* (2006.01) , *A61P 35/00* (2006.01) ,
C07K 14/47 (2006.01) , *C07K 7/06* (2006.01), *C07K 7/08* (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

PubMed, Delphion, CAPLus, Biosis, GenomeQuest

Keywords: YB-1, Akt, PKB, consensus site, phosphorylation site, RKYLRs, SEQ ID NO:11

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SUTHERLAND, B.W. et al. Akt phosphorylates the Y-box binding protein 1 at Ser102 located in the cold shock domain and affects the anchorage-independent growth of breast cancer cells. <i>Oncogene</i> . June 16, 2005, Vol. 24, No. 26, pages 4281-4292, ISSN 0950-9232. see whole document	1-41
Y	WO2005/117992 A2 (NEUMAN, T.) December 15, 2005. see whole document	1-41

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

27 June 2007 (27-06-2007)

Date of mailing of the international search report

26 July 2007 (26-07-2007)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Rena Oulton 819- 997-4567

INTERNATIONAL SEARCH REPORTInternational application No.
PCT/CA2007/000526**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. ☒ Claim Nos. : 21-36
because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 21 to 36 encompass a method of treatment of the human/animal body which this Authority is not obliged to search under Rule 39.1(iv) of the PCT, the search has been carried out based on the alleged effects of the compounds referred to therein.
2. ☐ Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :
3. ☐ Claim Nos. :
because they are dependant claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CA2007/000526

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2005117992	15-12-2005	US2006014712 A1	19-01-2006
		WO2005117928 A1	15-12-2005
