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(54) **Title**: METHODS FOR DETERMINING AGENTS TARGETING MENA ISOFORMS AND USES THEREOF FOR DIAGNOSIS AND TREATMENT OF METASTATIC TUMORS

(57) **Abstract**: The present invention relates to methods of determining agents that inhibit Mena^{+} or Mena$^{INV/+}$, and uses of agents that bind to and/or inhibit Mena^{+} or Mena$^{INV/+}$ for diagnosis and treatment of metastatic tumors.

METHODS FOR DETERMINING AGENTS TARGETING MENA ISOFORMS AND USES THEREOF FOR DIAGNOSIS AND TREATMENT OF METASTATIC TUMORS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 61/336,929, filed January 27, 2010, the content of which is hereby incorporated by reference into the subject application.

STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with government support under grant numbers 1-U54-CA112967, GM58801, GM38511, CA100324, and CA113395 awarded by the National Institutes of Health, U.S. Department of Health and Human Services. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0003] The present invention relates to methods of determining and using agents that inhibit $Mena^{+}$ or $Mena^{INV/+}$ for diagnosis and treatment of metastatic tumors.

BACKGROUND OF THE INVENTION

[0004] Throughout this application various publications are referred to in parenthesis. Full citations for these references may be found at the end of the specification. The disclosures of these publications are hereby incorporated by reference in their entirety into the subject application to more fully describe the art to which the subject invention pertains.

[0005] Mena is a cytoskeletal protein and is a member of the Ena/VASP family of proteins. These proteins are regulatory molecules which control cell movement, motility and shape in a number of cell types and organisms. They prevent the actin filaments from being capped by capping proteins at their barbed ends, amplifying the barbed end output and increasing metastatic potential in many tumors. Ena/VASP proteins are also constituents of the adherence junctions necessary to seal membranes in the epithelial sheet and control actin organization on cadherin adhesion contact. This process is frequently perturbed in cancer. Mena is upregulated in mouse and rat invasive breast cancer cells and overexpressed in human breast, colon, pancreatic, cervical and lung cancers. There are a number of isoforms, or splice variants, of Mena which are differentially expressed in

primary tumors, invasive cancer cells and metastases (1, 2). The broadly expressed form of the protein is referred to as "Mena". Mena^{11a} includes the "11a" exon and is found in primary tumors and sometimes in metastases, but not in invasive and metastatic cells during dissemination as Mena^{11a} is downregulated in invasive tumor cells (1). Mena^{+} is the Mena with the "+" exon. Mena$^{INV/+}$ has both the "+" exon and the "+++" exon (the "+++" exon is referred to here as the "INV" exon). Mena^{INV} is expressed only in invasive and metastatic cancer cells and not in primary tumors (1-3). Mena^{INV} is not observed in cells of adult animals that are not invasive cancer cells. Additionally, the "INV" exon sequence has no similarity to publicly known molecules. The presence of a hydrophobic cluster of residues in the C-terminal part of the sequence allows for a putative agent that is hydrophobic and membrane permeable. In adult animals, the "+" exon is normally only found in the central nervous system and cancer cells.

[0006] Expression of Mena^{INV} in mammary caricinoma cells increases lung metastases after injection into mammary fat pads. Mena^{INV}-cells exhibit increased *in vivo* cell motility rates and enhanced epidermal growth factor (EGF) chemotactic responses both *in vivo* and *in vitro* (3, 4). The Mena^{INV} cells also exhibit resistance to the epidermal growth factor receptor (EGFR) inhibitor TARCEVA® (erlotinib) both *in vitro* and *in vivo*. Compared to controls, cells expressing Mena^{INV} or Mena$^{INV/+}$ are capable of responding to 40- or 250-fold lower EGF concentrations, respectively (3). Conversely, cells expressing Mena^{11a} exhibit reduced responses to EGF both in vitro and in vivo (3). Therefore, switching of Mena isoforms results in dramatically altered motility responses to EGF and represents a mechanism that changes the sensitivity of invasive tumor cells to inhibitors of EGFR signaling (3). Analysis of signaling pathways downstream of EGFR indicates that canonical targets, such AKT and Erk and others involved in EGF-dependent proliferation are not affected by the Mena isoforms. Therefore, the effect of Mena isoforms on EGFR responses involves non-cannoncial pathways related to motility, chemotaxis and metastasis (3, 4). The risk of tumor cells undergoing metastasis increases with an increase in density of occurrences of an endothelial cell, a macrophage, and an invasive tumor cell in direct apposition in the tumor (4).

[0007] Once tumor cells have metastasized and established secondary tumors, survival rate decreases. Therefore, a metastasis inhibitor is sorely needed. The present invention advances this need by providing a method of determining chemotherapeutic agents that target Mena^{INV} and Mena$^{INV/+}$.

SUMMARY OF THE INVENTION

[0008] The present invention provides a method for determining a putative agent that binds to $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting Mena+ or $Mena^{INV/+}$ with the putative agent and measuring bound or unbound $Mena^{+}$ or $Mena^{INV/+}$.

[0009] The present invention also provides a method for determining a putative agent that inhibits $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent in the presence of a receptor tyrosine kinase-substrate gradient, and measuring actin polymerization or cell protrusion activity, wherein a decrease in or absence of actin polymerization or cell protrusion activity indicates inhibition of $Mena^{+}$ or $Mena^{INV/+}$.

[0010] The present invention further provides a method for determining a putative agent that inhibits metastasis of tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ *in vivo*, the method comprising contacting the $Mena^{+}$ or $Mena^{INV/+}$ expressing tumor with the putative agent, and measuring tumor metastasis.

[0011] The present invention additionally provides a method of treating a subject with a tumor expressing $Mena^{+}$ or $Mena^{INV/+}$, the method comprising administering to the subject a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor in an amount effective to treat the tumor.

[0012] The present invention also provides a method for determining a putative agent that inhibits metastasis of a tumor, the method comprising contacting the putative agent with a cell line or tissue culture that expresses $Mena^{+}$ or $Mena^{INV/+}$, wherein reduction in the expression of $Mena^{+}$ or $Mena^{INV/+}$ is indicative that the putative agent is a candidate for inhibiting metastasis of a tumor or wherein lack of reduction in the expression of $Mena^{+}$ or $Mena^{INV/+}$ is indicative that the compound is not a candidate compound for inhibiting metastasis of a tumor.

[0013] The present invention provides the putative agent identified by the method for (1) determining a putative agent that binds to $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting $Mena^{+}$ or $Mena^{INV/+}$ and measuring bound or unbound $Mena^{+}$ or $Mena^{INV/+}$; (2) determining a putative agent that inhibits $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent in the presence of a receptor tyrosine kinase-substrate gradient, and measuring actin polymerization or cell protrusion activity, wherein a decrease in or absence of actin polymerization or cell protrusion activity indicates inhibition of $Mena^{+}$ or $Mena^{INV/+}$; or (3) determining a putative agent that inhibits metastasis of tumor cells

expressing $Mena^{+}$ or $Mena^{INV/+}$ *in vivo*, the method comprising contacting the $Mena^{+}$ or $Mena^{INV/+}$ expressing tumor with the putative agent, and measuring tumor metastasis.

[0014] The present invention provides a pharmaceutical composition comprising a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor formulated in dosage form for treating a tumor.

[0015] The present invention also provides the use of a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor for the treatment of a tumor. The present invention further provides the use of a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor for the preparation of a medicament for the treatment of a tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] Figure 1A-1B. (A) Mena sequence showing location of INV and + exons. (B) *In vitro* sensitivity of Mena, $Mena^{INV}$, $Mena^{INV/+}$ and GFP control to various EGF concentrations. Protrusion of starved cells 3 minutes after EGF addition. GFP control expresses no MENA isoform. Control drops to background level around 0.5nM. $MENA^{INV/+}$ can respond to 2 orders of magnitude lower EGF concentration, compared to control. * $p < 0.05$ vs. GFP control.

[0017] Figure 2A-2B. $Mena^{INV}$ promotes invasion of cells *in vivo* assay. (A) Isoform of Mena expressed affects response to EGF gradient. Optimal response of each isoform is dependent on EGF concentration. $Mena^{INV}$ response peaks around 1nM, Mena response peaks around 25nM. (B) Graphic of *in vivo* assay with needle collection. Primary tumor may be xenograft.

[0018] Figure 3A-3C. $Mena^{INV}$ cells are less sensitive to inhibition by erlotinib and still participate in the paracrine loop during *in vivo* invasion. Collection needle contained both EGF and erlotinib. MTLn3-EGFP cells are control. ** $p < 0.01$, *** $p < 0.001$.

[0019] Figure 4A-4D. Mena isoform containing both + and INV exons in the same transcript is expressed in needle collected cells from PyMT tumor model (1). (A) Diagram of Mena domain organization with primers. (B-D) APTC - average primary tumor cells. NC - needle collected cells. +plasm - plasmid of the Mena + exon. +++plasm - exon of the Mena INV exon.

[0020] Figure 5. Kaplan Meir survival curves of PyMT Mena transgenic animals.

[0021] Figure 6. *In vivo* invasion assay of PyMT mena transgenic animals. ** $p < 0.01$, *** $p < 0.005$.

DETAILED DESCRIPTION OF THE INVENTION

[0022] The present invention provides a method for determining a putative agent that binds to $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent and measuring bound or unbound $Mena^{+}$ or $Mena^{INV/+}$, wherein a increase in $Mena^{+}$ or $Mena^{INV/+}$ bound to the agent or a decrease in unbound $Mena^{+}$ or $Mena^{INV/+}$ in the presence of the agent indicates that the agent binds to $Mena^{+}$ or $Mena^{INV/+}$. $Mena^{+}$ or $Mena^{INV/+}$, for example, can be applied to an artificial substrate to screen for agents that bind to these Mena isoforms *in vitro*.

[0023] The present invention also provides a method for determining a putative agent that inhibits $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent in the presence of a receptor tyrosine kinase-substrate gradient, and measuring actin polymerization or cell protrusion activity, wherein a decrease in or absence of actin polymerization or cell protrusion activity in the presence of the agent indicates inhibition of $Mena^{+}$ or $Mena^{INV/+}$ and wherein a lack of decrease in actin polymerization or cell protrusion activity indicates lack of inhibition of $Mena^{+}$ or $Mena^{INV/+}$.

[0024] Tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ can be used to screen for agents that inhibit the ability of $Mena^{+}$ or $Mena^{INV/+}$ to sensitize tumor cells to low concentrations of epidermal growth factor (EGF) that, in the absence of these Mena isoforms, do not normally stimulate action polymerization and/or cell protrusion activity.

[0025] Cells expressing the different Mena isoforms can be used to identify inhibitors of EGF-dependent motility/chemotaxis and dissemination of tumor cells from the primary tumor. Expression of $Mena^{INV}$ or $Mena^{INV/+}$ renders cells refractory to TARCEVA® (erlotinib) and other EGFR inhibitors while potentiating their response to EGF. Therefore, cells expressing the different Mena isoforms can be used to screen for inhibitors that target the components of the EGF response that are specific to motility and metastasis-related responses. Such a screen can use the increased lamellipodial protrusion or enhanced actin polymerization observed in cells expressing $Mena^{INV}$ or $Mena^{INV/+}$ after treatment with low EGF concentrations. Differential mass spectrometry approaches such as SILAC may be used to compare $Mena^{INV}$ or $Mena^{INV/+}$ with Mena11a to identify targets that distinguish between the enhanced or suppressed EGF responses in cells expressing different Mena isoforms.

[0026] The present invention additionally provides a method for determining a putative agent that inhibits metastasis of a tumor, the method comprising contacting the putative agent with a cell line or tissue culture that expresses $Mena^{+}$ or $Mena^{INV/+}$, wherein reduction in the expression of $Mena^{+}$ or $Mena^{INV/+}$ in the presence of the agent is indicative that the putative agent is a candidate for inhibiting metastasis of a tumor or wherein lack of reduction in the expression of $Mena^{+}$ or $Mena^{INV/+}$ is indicative that the agent is not a candidate for inhibiting metastasis of a tumor.

[0027] The present invention further provides a method for determining a putative agent that inhibits metastasis of tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ *in vivo*, the method comprising contacting the $Mena^{+}$ or $Mena^{INV/+}$ expressing tumor with the putative agent, and measuring tumor metastasis. Tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ can be transplanted into experimental animals, such as for example mice, to form tumors. Agents can be screened for their ability to inhibit metastasis from these tumors.

[0028] The invention provides a method for determining whether a subject has a metastatic tumor comprising assaying a blood, tissue and/or tumor sample of the subject for expression of $Mena^{+}$ and/or $Mena^{INV/+}$, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ indicates the presence of a metastatic tumor.

[0029] The invention also provides a method for determining whether a subject has a metastatic tumor comprising assaying a blood, tissue and/or tumor sample of the subject for expression of Menal1a, and $Mena^{+}$ and/or $Mena^{INV/+}$, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ and decreased expression of Menal1a together indicates the presence of a metastatic tumor.

[0030] The invention provides a method for assessing the efficacy of therapy to treat a metastatic tumor in a subject who has undergone or is undergoing treatment for a metastatic tumor, the method comprising assaying a blood, tissue and/or tumor sample of the subject for expression of $Mena^{+}$ and/or $Mena^{INV/+}$, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ is indicative of a need to continue therapy to treat the tumor.

[0031] The invention also provides a method for assessing the efficacy of therapy to treat a metastatic tumor in a subject who has undergone or is undergoing treatment for a metastatic tumor, the method comprising assaying a blood, tissue and/or tumor sample of the subject for expression of Menal1a, and $Mena^{+}$ and/or $Mena^{INV/+}$, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ and decrease in expression of Menal1a is indicative of a need to continue therapy to treat the tumor.

[0032] The invention further provides a method for assessing the prognosis of a subject who has a metastatic tumor, comprising assaying a blood, tissue and/or tumor sample of the subject for expression of $Mena^{+}$ and/or $Mena^{INV/+}$, wherein the subject's prognosis improves with a decrease in expression of $Mena^{+}$ and/or $Mena^{INV/+}$.

[0033] The invention further also provides a method for assessing the prognosis of a subject who has a metastatic tumor, comprising assaying a blood, tissue and/or tumor sample of the subject for expression of Mena11a, and $Mena^{+}$ and/or $Mena^{INV/+}$, wherein the subject's prognosis improves with a decrease in expression of $Mena^{+}$ and/or $Mena^{INV/+}$, and an increase in expression of Mena11a.

[0034] As used herein, changes in the expression of $Mena^{+}$, $Mena^{INV/+}$ and Mena11a mean changes in expression relative to their levels in normal tissue or relative to their levels in *in situ* (non-metastatic) carcinomas. The expression of $Mena^{+}$, $Mena^{INV/+}$ and Mena11a can be normalized relative to the expression of protein variants that are not changed in expression in a metastatic tumor. Examples of proteins that could be used as controls include those of the Ena/VASP family that are unchanged in their expression in metastatic cells, including the 140K and 80K isoforms of Mena, and VASP. Other examples of proteins or genes that could be used as controls include those listed as relatively unchanged in expression such as N-WASP, Rac1, Pak1, and PKCalpha and beta. Preferred controls include the 80K and 140K isoforms of Mena and VASP. The expression of $Mena^{+}$or $Mena^{INV/+}$ can be compared to expression of Mena 11a, i.e. Mena+/Mena11a expression ratio or $Mena^{INV/+}$/Mena11a expression ratio.

[0035] The expression of $Mena^{+}$, $Mena^{INV/+}$ and Mena11a may be detected in *vitro* or *in vivo*. The expression may be detected at the level of the nucleic acid variant and/or at the level of the protein isoform. Where expression is detected *in vitro*, a sample of blood, tumor, tissue or cells from the subject may be removed using standard procedures, including biopsy and aspiration. Cells which are removed from the subject may be analyzed using immunocytofluorometry (FACS analysis). The expression of $Mena^{+}$, $Mena^{INV/+}$ and Mena11a may be detected by detection methods readily determined from the known art, including, without limitation, immunological techniques such as Western blotting, hybridization analysis, fluorescence imaging techniques, and/or radiation detection.

[0036] The invention provides a method of inhibiting metastasis of a tumor in a subject, the method comprising reducing the presence or activity of $Mena^{+}$ or $Mena^{INV/+}$ in the subject. The invention also provides a method of inhibiting metastasis of a tumor in a

subject, the method comprising reducing the presence or activity of $Mena^{+}$ or $Mena^{INV/+}$, and increasing the presence or activity of Mena 11a in the subject.

[0037] The methods can involve intervention at the level of DNA, RNA, and/or protein. For example, the presence or activity of the isoform can be reduced by addition of an antisense molecule, a ribozyme, or an RNA interference (RNAi) molecule to the tumor, where the antisense molecule, ribozyme or RNAi molecule specifically inhibits expression of the isoform. The antisense molecule, ribozyme, or RNAi molecule can be comprised of nucleic acid (e.g., DNA or RNA) or nucleic acid mimetics (e.g., phosphorothionate mimetics) as are known in the art. Methods for treating tissue with these compositions are also known in the art. The antisense molecule, ribozyme or RNAi molecule can be added directly to the cancerous tissue in a pharmaceutical composition that preferably comprises an excipient that enhances penetration of the antisense molecule, ribozyme or RNAi molecule into the cells of the tissue. The antisense molecule, ribozyme or RNAi can be expressed from a vector that is transfected into the cancerous tissue. Such vectors are known in the art.

[0038] The presence or activity of the isoform can be reduced by addition of an antibody or aptamer to the tissue, wherein the antibody or aptamer specifically binds to and reduces the activity of the isoform in the tissue. The antibody or aptamer can be added directly to the tissue, preferably in a pharmaceutical composition comprising an agent that enhances penetration of the antibody or aptamer into the tissue. The antibody or aptamer can be encoded on a vector that is used to transfect the cancerous tissue.

[0039] The invention provides kits for detecting the presence or absence of a metastatic tumor, where the kits comprise an antibody, a peptide or an aptamer that specifically binds to $Mena^{+}$ or $Mena^{INV/+}$ isoforms, and/or a probe or PCR primers that specifically hybridize to nucleic acid encoding the $Mena^{+}$ or $Mena^{INV/+}$ isoforms. The kits can additionally comprise an agent for detecting the presence or absence of Mena11a.

Table 1. Human Mena Sequences

Mena+

APSSDSSLSS APLPEYSSCQ PPSAPPPSYA KVISAPVSDA TPDYAVVTAL PPTSTPPTPP LRHAATRFAT SLGSAFHPVL PHYATVPRPL NKNSRPSSPV NTPSSQPPAA KSCAQPTSNF SPLPPSPPIM ISSPPGKATG PRPVLPVCVS SPVPQMPPSP TAPNGSLDSV TYPVSPPPTS GPAAPPPPPP PPPPPPPPPL PPPPLPPLAS LSHCSGSQASP PPGTPLASTP SSKPSVLPSP SAGAPA (SEQ ID NO:1)

Mena ++

FYLG (SEQ ID NO:2)

ttctatttag gg (SEQ ID NO:5)

$Mena^{INV}$ (Mena +++)
AQSKVTATQD STNLRCIFC (SEQ ID NO:3)
gcccagagca aggttactgc tacccaggac agcactaatt tgcgatgtat tttctgt (SEQ ID NO:6)

Mena 11a
RDSPRKNQIV FDNRSYDSLH R (SEQ ID NO:4)
acgggattct ccaaggaaaa atcagattgt ttttgacaac aggtcctatg attcattaca cag (SEQ ID NO:7)

[0040] An agent that specifically binds to $Mena^{+}$ or $Mena^{INV/+}$ or Mena11a can be labeled with a detectable marker. Labeling may be accomplished using one of a variety of labeling techniques, including peroxidase, chemiluminescent, and/or radioactive labels known in the art. The detectable marker may be, for example, a nonradioactive or fluorescent marker, such as biotin, fluorescein (FITC), acridine, cholesterol, or carboxy-X-rhodamine, which can be detected using fluorescence and other imaging techniques readily known in the art. Alternatively, the detectable marker may be a radioactive marker, including, for example, a radioisotope. The radioisotope may be any isotope that emits detectable radiation, such as, for example, ^{35}S, ^{32}P, or ^{3}H. Radioactivity emitted by the radioisotope can be detected by techniques well known in the art. For example, gamma emission from the radioisotope may be detected using gamma imaging techniques, particularly scintigraphic imaging.

[0041] The expression of $Mena^{+}$ or $Mena^{INV/+}$ or Mena11a in a subject may be detected through hybridization analysis of nucleic acid extracted from a blood, tumor, tissue or cell sample from the subject using one or more nucleic acid probes which specifically hybridize to nucleic acid encoding $Mena^{+}$ or $Mena^{INV/+}$ or Mena11a. The nucleic acid probes may be DNA or RNA, and may vary in length from about 8 nucleotides to the entire length of the ++ or +++ nucleic acid variant of Mena. Hybridization techniques are well known in the art. The probes may be prepared by a variety of techniques known to those skilled in the art, including, without limitation, restriction enzyme digestion of Mena nucleic acid; and automated synthesis of oligonucleotides whose sequence corresponds to selected portions of the nucleotide sequence of the Mena nucleic acid, using commercially-available oligonucleotide synthesizers, such as the Applied Biosystems Model 392 DNA/RNA synthesizer.

[0042] The nucleic acid probes may be labeled with one or more detectable markers. Labeling of the nucleic acid probes may be accomplished using a number of methods known in the art (*e.g.*, nick translation, end labeling, fill-in end labeling, polynucleotide

kinase exchange reaction, random priming, or SP6 polymerase) with a variety of labels (e.g., radioactive labels, such as ^{35}S, ^{32}P, or ^{3}H, or nonradioactive labels, such as biotin, fluorescein (FITC), acridine, cholesterol, or carboxy-X-rhodamine (ROX)).

[0043] A putative agent that binds to $Mena^{+}$ or $Mena^{INV/+}$ protein is likely to be a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor when administered to cancer cells. Therefore, inhibitors of the Mena isoforms will inhibit receptor tyrosine kinase-substrate-dependent motility/chemotaxis of cancer cells expressing the Mena isoforms and will inhibit dissemination of tumor cells from the primary tumor.

[0044] Receptor tyrosine kinases are high affinity cell surface receptors for many polypeptides, growth factors, and hormones. Receptor tyrosine kinase-substrates include many polypeptides, growth factors and hormones known in the art. Any of the receptor tyrosine kinase-substrates known in the art may be used to create a chemotactic gradient. For example, EGF or PDGF may be used to establish a chemotactic grandient to stimulate cell motility and chemotaxis.

[0045] Actin polymerization is necessary in chemotaxis and cytokinesis. A chemical gradient, such as a receptor tyrosine kinase-substrate gradient, results in the polymerization of actin filaments within eukaryote cells. The actin filaments are polymerized with the barbed/growing ends of the actin polymerizing towards the chemical gradient. $Mena^{+}$ or $Mena^{INV/+}$ prevent the capping of the growing actin polymer chains, leading to continued actin polymerization. Actin polymerization results in the creation of cell protrusions, eventually resulting in chemotaxis. Preventing the capping of actin filaments results in heightened cell protrusion activity and therefore, heightened cell motility/metastasis.

[0046] Expression of $Mena^{+}$ or $Mena^{INV/+}$ renders cells refractory to TARCEVA® (erlotinib) and other EGFR inhibitors while potentiating their response to EGF. $Mena^{INV/+}$ cells respond to EGF concentrations two orders of magnitude lower than cells which do not express $Mena^{INV/+}$. Cells that express $Mena^{+}$ or $Mena^{INV/+}$ will chemotax up an EGF gradient. Since $Mena^{+}$ or $Mena^{INV/+}$ potentiates the cell's response to EGF, cancer cells expressing $Mena^{+}$ or $Mena^{INV/+}$ will respond to EGF gradients that cells which are not expressing $Mena^{+}$ or $Mena^{INV/+}$ will not respond to.

[0047] Actin polymerization or cell protrusion activity can be measured by any method known in the art including, but not limited to, microscopy, molecular imaging, and live cell imaging. A decrease in the level of actin polymerization or cell protrusion activity of the cancer cells in the presence of an EGF gradient after contacting the cells with the putative

agent compared with the level of actin polymerization or cell protrusion activity of the cancer cells in the presence of an EGF gradient before contacting the cells with the putative agent indicates that the putative agent is an inhibitor of $Mena^{+}$ or $Mena^{INV/+}$. A decrease in the level of actin polymerization or cell protrusion activity of the cancer cells is any decrease, from a statistically significant lessening in the level of actin polymerization or cell protrusion activity through a total absence of actin polymerization or cell protrusion activity after contacting the cells with the putative agent. Actin polymerization or cell protrusion activity can also be measured *in vitro* by any method known in the art including, but not limited to, assaying invasion of cells in a collagen gel.

[0048] The method for determining a putative agent that inhibits $Mena^{+}$ or $Mena^{INV/+}$ may further comprise at least one of the following controls: (1) measuring actin polymerization or cell protrusion activity of cancer cells expressing $Mena^{+}$ or $Mena^{INV/+}$ in the presence of an EGF gradient ; or (2) contacting cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent in the presence of an EGF gradient that would, even in the absence of $Mena^{+}$ or $Mena^{INV/+}$, stimulate actin polymerization or cell protrusion activity, and measuring actin polymerization or cell protrusion activity. Both controls (1) and (2) will show actin polymerization or cell protrusion activity. Control (2) will show actin polymerization or cell protrusion activity even when the putative agent is an inhibitor of $Mena^{+}$ or $Mena^{INV/+}$.

[0049] Tumor metastasis can be measured by any method known in the art including, but not limited to, an *in vivo* invasion assay, a modified Boyden chamber assay or capture of cells migrating into a catheterized needle. The *in vivo* invasion assay may be done by any method in the art including, but not limited to, insertion of a needle with EGF into the tumor, with needle collection of cells. Alternatively, tumor metastasis can be measured *in vivo* by any imaging method known in the art such as intravital imaging of tumors. Tumor mestatsis may also be measured by any method of imaging cells known in the art such as by high-resolution microscopy, multiphoton imaging or low resolution microscopy with staining. The tumor cells can be obtained by any method known in the art, including tumors derived from injecting a subject with cells expressing $Mena^{+}$ or $Mena^{INV/+}$. Since cells expressing $Mena^{+}$ or $Mena^{INV/+}$ will move up an EGF gradient, when the cells expressing $Mena^{+}$ or $Mena^{INV/+}$ are *in vivo*, the cells expressing $Mena^{+}$ or $Mena^{INV/+}$ will move up the imposed EGF gradient and can be needle collected. If the putative agent does in fact inhibit $Mena^{+}$ or $Mena^{INV/+}$, the sensitivity of cells expressing $Mena^{+}$ or $Mena^{INV/+}$ drops and fewer

cells will move up the EGF gradient. Therefore, the collection of fewer cells $Mena^{+}$ or $Mena^{INV/+}$ by the *in vivo* invasion assay after contacting said cells with the putative agent indicates that the putative agent does in fact inhibit $Mena^{+}$ or $Mena^{INV/+}$.

[0050] If a subject has a tumor expressing $Mena^{+}$ or $Mena^{INV/+}$, the putative agent can be administered and metastasis of the cancer cells can be measured. Alternatively, cancer cells expressing $Mena^{+}$ or $Mena^{INV/+}$ can be implanted into a subject to form tumors. Mestastasis of cancer cells from these tumors may be measured by any method known in the art including *in vivo* assay, and measuring the number of tumor cell's in the subject's blood or lymphatic vessels.

[0051] The subject can be any mammal, such as a rodent or a human.

[0052] The present invention provides a method of treating a subject with a tumor expressing $Mena^{+}$ or $Mena^{INV/+}$, the method comprising administering to the subject a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor in an amount effective to treat the tumor.

[0053] Treating a subject's tumor means inhibiting the $Mena^{+}$ or $Mena^{INV/+}$ expressed by the subject's cancer cells or inhibiting the subject's tumor from metastasizing. The amount of the putative agent effective to treat the tumor will vary depending on the type of tumor, the size and severity of the tumor, and the subject's physiology. Appropriate amounts of the putative agent effective to treat the tumor can be readily determined by the skilled artisan without undue experimentation. The manner of administration of the putative agent depends on the type and site of the tumor. According to the methods of the present invention, the putative agent may be administered by any method known in the art, including but not limited to, oral or parenteral administration.

[0054] The putative agent can be a small molecule, an antibody, a peptide, a protein, a protein fragment or an aptamer. Preferably, the putative agent is hydrophilic and membrane permeable.

[0055] The tumor cell expressing $Mena^{+}$ or $Mena^{INV/+}$ can be a breast, pancreas, prostate, colon, brain, liver, lung, head or neck tumor cell or can be a secretory epithelial tumor cell.

[0056] The present invention provides the putative agent identified by the method for (1) determining a putative agent that binds to $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting $Mena^{+}$ or $Mena^{INV/+}$ and measuring bound or unbound $Mena^{+}$ or $Mena^{INV/+}$; (2) determining a putative agent that inhibits $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the

putative agent in the presence of a receptor tyrosine kinase-substrate gradient, and measuring actin polymerization or cell protrusion activity, wherein a decrease in or absence of actin polymerization or cell protrusion activity indicates inhibition of $Mena^{+}$ or $Mena^{INV/+}$; or (3) determining a putative agent that inhibits metastasis of tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ *in vivo*, the method comprising contacting the $Mena^{+}$ or $Mena^{INV/+}$ expressing tumor with the putative agent, and measuring tumor metastasis.

[0057] The present invention also provides a pharmaceutical composition comprising a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor formulated in dosage form for treating a tumor. The formulation of the pharmaceutical composition in a dosage form for treating a tumor comprises the $Mena^{+}$ or $Mena^{INV/+}$ inhibitor in a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier used will depend on the method of administration as well as the subject to whom the pharmaceutical composition will be administered. Any pharmaceutically acceptable carrier known in the art can be used.

[0058] For oral administration, the formulation of the $Mena^{+}$ or $Mena^{INV/+}$ inhibitor may be presented as capsules, tablets, powder, granules, or as a suspension. The formulation may have conventional additives, such as lactose, mannitol, corn starch, or potato starch. The formulation may also be presented with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch, or gelatins. Additionally, the formulation may be presented with disintegrators, such as corn starch, potato starch, or sodium carboxymethylcellulose. The formulation also may be presented with dibasic calcium phosphate anhydrous or sodium starch glycolate. Finally, the formulation may be presented with lubricants, such as talc or magnesium stearate.

[0059] For a parenteral administration, the $Mena^{+}$ or $Mena^{INV/+}$ inhibitor may be combined with a sterile aqueous solution which is preferably isotonic with the blood of the subject. Such a formulation may be prepared by dissolving a solid active ingredient in water containing physiologically-compatible substances, such as sodium chloride, glycine, and the like, and having a buffered pH compatible with physiological conditions, so as to produce an aqueous solution, then rendering said solution sterile. The formulations may be present in unit or multi-dose containers, such as sealed ampoules or vials. The formulation may be delivered by any mode of injection, including, without limitation, epifascial, intrasternal, intravascular, intravenous, parenchymatous, or subcutaneous.

[0060] The present invention provides for the use of a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor for the treatment of a tumor. The present invention further provides for the use of a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor for the preparation of a medicament for the treatment of tumor.

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2. Gertler FB, Niebuhr K, Reinhard M, Wehland J, Soriano P. Mena, a Relative of VASP and Drosophila Enabled, Is Implicated in the Control of Microfilament Dynamics. Cell 87: 227-239 Oct. 18, 1996.
3. Philippar U, Roussos ET, Oser M, Yamaguchi H, Kim HD, Giampieri S, Wang Y, Goswami S, Wyckoff JB, Lauffenburger DA, Sahai E, Condeelis JS, Gertler FB. A Mena Invasion Isoform Potentiates EGF-Induced Carcinoma Cell Invasion and Metastasis. Developmental Cell 15: 813-828 Dec. 9, 2008.
4. Robinson BD, Sica GL, Liu YF, Rohan TE, Gertler FB, Condeelis JS, Jones JG. Tumor Microenvironment of Metastasis in Human Breast Carcinoma: A Potential Prognostic Marker Linked to Hematogenous Dissemination. Clin Cancer Res 2009, 15(7): 2433-41; Epub 2009 Mar 24.
5. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124:263-6.

What is claimed is:

1. A method for determining a putative agent that inhibits $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent in the presence of a receptor tyrosine kinase-substrate gradient, and measuring actin polymerization or cell protrusion activity, wherein a decrease in or absence of actin polymerization or cell protrusion activity in the presence of the agent indicates inhibition of $Mena^{+}$ or $Mena^{INV/+}$ and wherein a lack of a decrease in actin polymerization or cell protrusion activity indicates a lack of inhibition of $Mena^{+}$ or $Mena^{INV/+}$.

2. The method of Claim 1, wherein the receptor tyrosine kinase-substrate gradient is one that, in the absence of $Mena^{+}$ or $Mena^{INV/+}$, would not stimulate actin polymerization or cell protrusion activity.

3. The method of Claim 1 or 2, wherein the receptor tyrosine kinase-substrate is EGF.

4. The method of any of Claims 1 to 3, further comprising at least one control.

5. The method of Claim 4, wherein the control comprises measuring actin polymerization or cell protrusion activity of tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ in the presence of a receptor tyrosine kinase-substrate gradient.

6. The method of Claim 4, wherein the control comprises contacting cells expressing $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent in the presence of a receptor tyrosine kinase-substrate gradient which, even in the absence of $Mena^{+}$ or $Mena^{INV/+}$ would stimulate actin polymerization or cell protrusion activity, and measuring actin polymerization or cell protrusion activity.

7. The method of any of Claims 1 to 6, wherein the tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ are *in vivo*.

8. The method of Claim 6, wherein measuring actin polymerization or cell protrusion activity comprises an *in vivo* invasion assay.

9. The method of Claim 8, wherein the collection of fewer cells by the *in vivo* invasion assay in the tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ which were contacted by the putative agent indicates that the putative agent inhibits $Mena^{+}$ or $Mena^{INV/+}$.

10. A method for determining a putative agent that inhibits metastasis of a tumor, the method comprising contacting the putative agent with a cell line or tissue culture that expresses $Mena^{+}$ or $Mena^{INV/+}$, wherein reduction in the expression of $Mena^{+}$ or $Mena^{INV/+}$ in the presence of the agent is indicative that the putative agent is a candidate for inhibiting metastasis of a tumor or wherein lack of reduction in the expression of $Mena^{+}$ or $Mena^{INV/+}$ is indicative that the agent is not a candidate for inhibiting metastasis of a tumor.

11. A method for determining a putative agent that inhibits metastasis of tumor cells expressing $Mena^{+}$ or $Mena^{INV/+}$ *in vivo*, the method comprising contacting the $Mena^{+}$ or $Mena^{INV/+}$ expressing tumor with the putative agent, and measuring tumor metastasis.

12. The method of Claim 11, wherein measuring tumor metastasis comprises using an *in vivo* invasion assay.

13. The method of Claim 12, wherein the *in vivo* invasion assay comprises collecting tumor cells migrating along a receptor tyrosine kinase-substrate gradient.

14. The method of Claim 13, wherein the receptor tyrosine kinase-substrate is EGF.

15. The method of Claim 11, wherein measuring tumor metastasis comprises analyzing the number of tumor cells in a subject's blood or lymphatic vessels.

16. A method of treating a subject with a tumor expressing $Mena^{+}$ or $Mena^{INV/+}$, the method comprising administering to the subject a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor in an amount effective to treat the tumor.

17. The method of any of Claims 7 to 9 or 11 to 16, wherein the subject is a mammal.

18. The method of Claim 17, wherein the subject is a rodent.

19. The method of Claim 18, wherein the subject is a human.

20. A method for determining a putative agent that binds to $Mena^{+}$ or $Mena^{INV/+}$, the method comprising the steps of contacting $Mena^{+}$ or $Mena^{INV/+}$ with the putative agent and measuring bound or unbound $Mena^{+}$ or $Mena^{INV/+}$, wherein a increase in $Mena^{+}$ or $Mena^{INV/+}$ bound to the agent or a decrease in unbound $Mena^{+}$ or $Mena^{INV/+}$ in the presence of the agent indicates that the agent binds to $Mena^{+}$ or $Mena^{INV/+}$.

21. The method of any of Claims 1 to 20, wherein the putative agent is a small molecule, an antibody, a peptide, a protein, a protein fragment or an aptamer.

22. The method of any of Claims 1 to 20, wherein a tumor cell expressing $Mena^{+}$ or $Mena^{INV/+}$ is a breast, pancreas, prostate, colon, brain, liver, lung, head or neck tumor cell.

23. The method of any of Claims 1 to 20, wherein a tumor cell expressing $Mena^{+}$ or $Mena^{INV/+}$ is a secretory epithelial tumor cell.

24. The method of Claim 16, wherein the $Mena^{+}$ or $Mena^{INV/+}$ inhibitor is administered orally.

25. The method of Claim 16, wherein the $Mena^{+}$ or $Mena^{INV/+}$ inhibitor is administered parenterally.

26. The putative agent identified by the method of any of Claims 1 to 15.

27. A pharmaceutical composition comprising a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor formulated in dosage form for treating a tumor.

28. The pharmaceutical composition of Claim 27, wherein the pharmaceutical composition is formulated for oral administration.

29. The pharmaceutical composition of Claim 27, wherein the pharmaceutical composition is formulated for parenteral administration.

30. A method for determining whether a subject has a metastatic tumor comprising assaying a blood, tissue and/or tumor sample of the subject for expression of $Mena^{+}$ and/or $Mena^{INV/+}$, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ indicates the presence of a metastatic tumor.

31. The method of Claim 30, which further comprises assaying the subject's blood, tissue and/or tumor sample for expression of Mena11a, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ and decreased expression of Mena11a together indicates the presence of a metastatic tumor.

32. A method for assessing the efficacy of therapy to treat a metastatic tumor in a subject who has undergone or is undergoing treatment for a metastatic tumor, the method comprising assaying a blood, tissue and/or tumor sample of the subject for expression of $Mena^{+}$ and/or $Mena^{INV/+}$, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ is indicative of a need to continue therapy to treat the tumor.

33. The method of Claim 32, which further comprises assaying the subject's blood, tissue and/or tumor sample for expression of Mena11a, wherein overexpression of $Mena^{+}$ and/or $Mena^{INV/+}$ and decrease in expression of Mena11a is indicative of a need to continue therapy to treat the tumor.

34. A method for assessing the prognosis of a subject who has a metastatic tumor, comprising assaying a blood, tissue and/or tumor sample of the subject for expression of $Mena^{+}$ and/or $Mena^{INV/+}$, wherein the subject's prognosis improves with a decrease in expression of $Mena^{+}$ and/or $Mena^{INV/+}$.

35. The method of Claim 34, which further comprises assaying the subject's blood, tissue and/or tumor sample for expression of Mena11a, wherein the subject's prognosis improves with a decrease in expression of $Mena^{+}$ and/or $Mena^{INV/+}$, and an increase in expression of Mena11a.

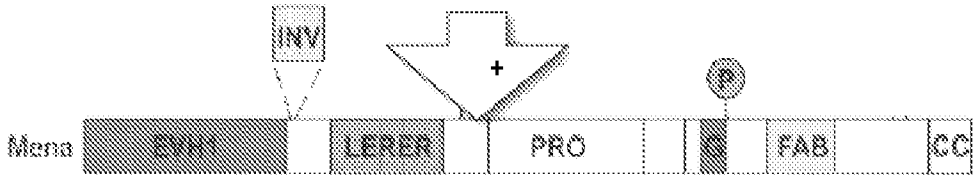
36. The method of any of Claims 30-35, wherein the tumor is a secretory epithelial tumor.

37. The method of any of Claims 30-35, wherein the tumor is a breast, pancreas, prostate, colon, brain, liver, lung, head or neck tumor.

38. Use of a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor for the treatment of a tumor.

39. Use of a $Mena^{+}$ or $Mena^{INV/+}$ inhibitor for the preparation of a medicament for the treatment of a tumor.

A



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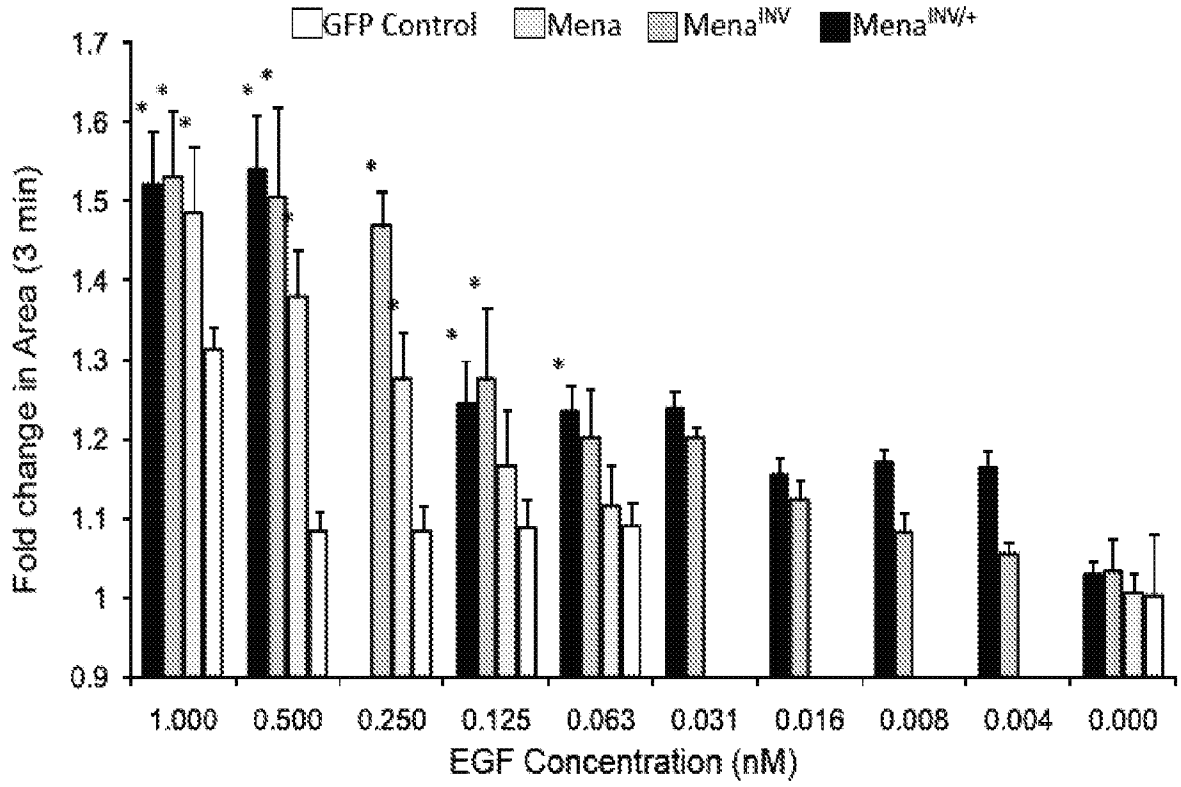


FIGURE 1A-1B

A

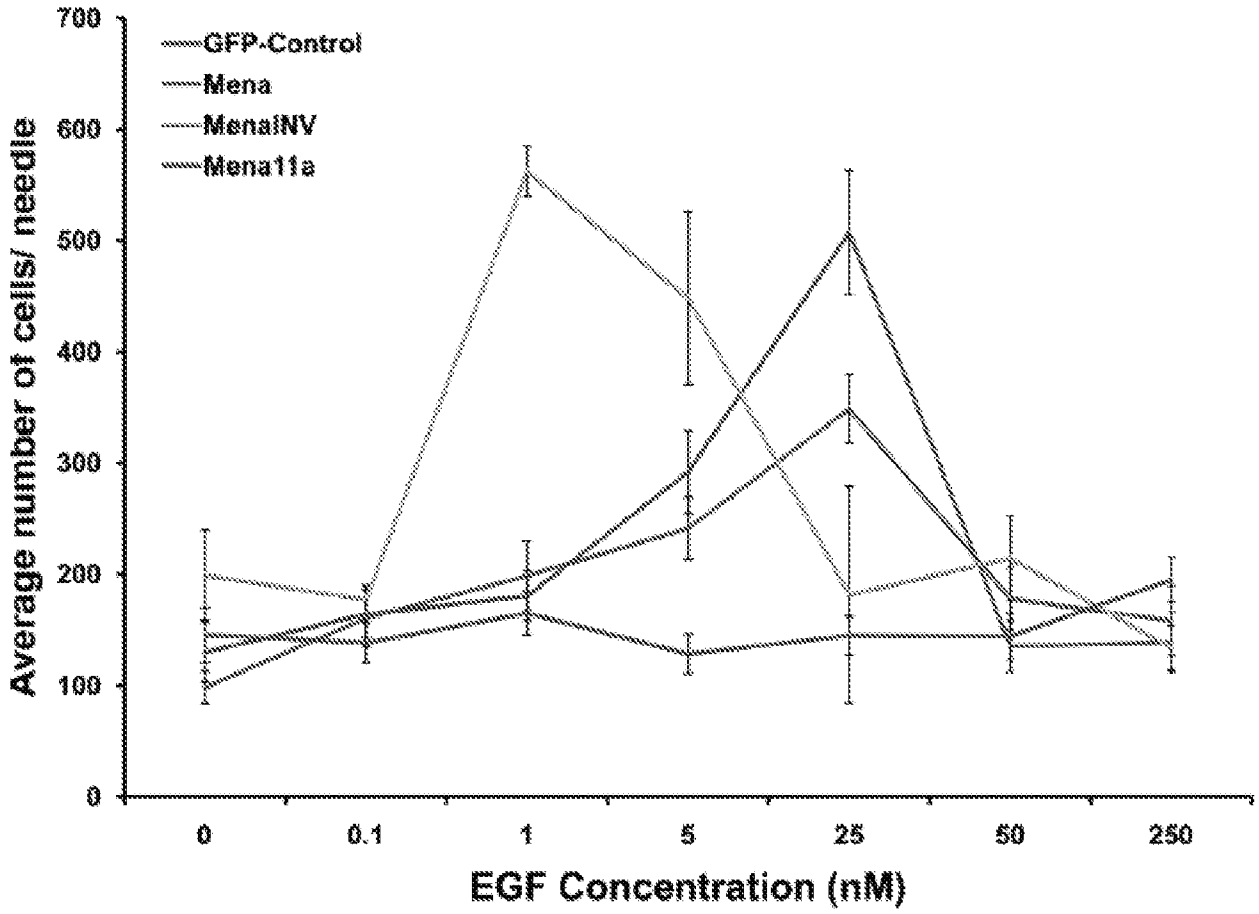

GFP-Control
Mena
MenaINV
Mena11a
Average number of cells/ needle
700
600
500
400
300
200
100
0
0
0.1
1
5
25
50
250
EGF Concentration (nM)

FIGURE 2A

B

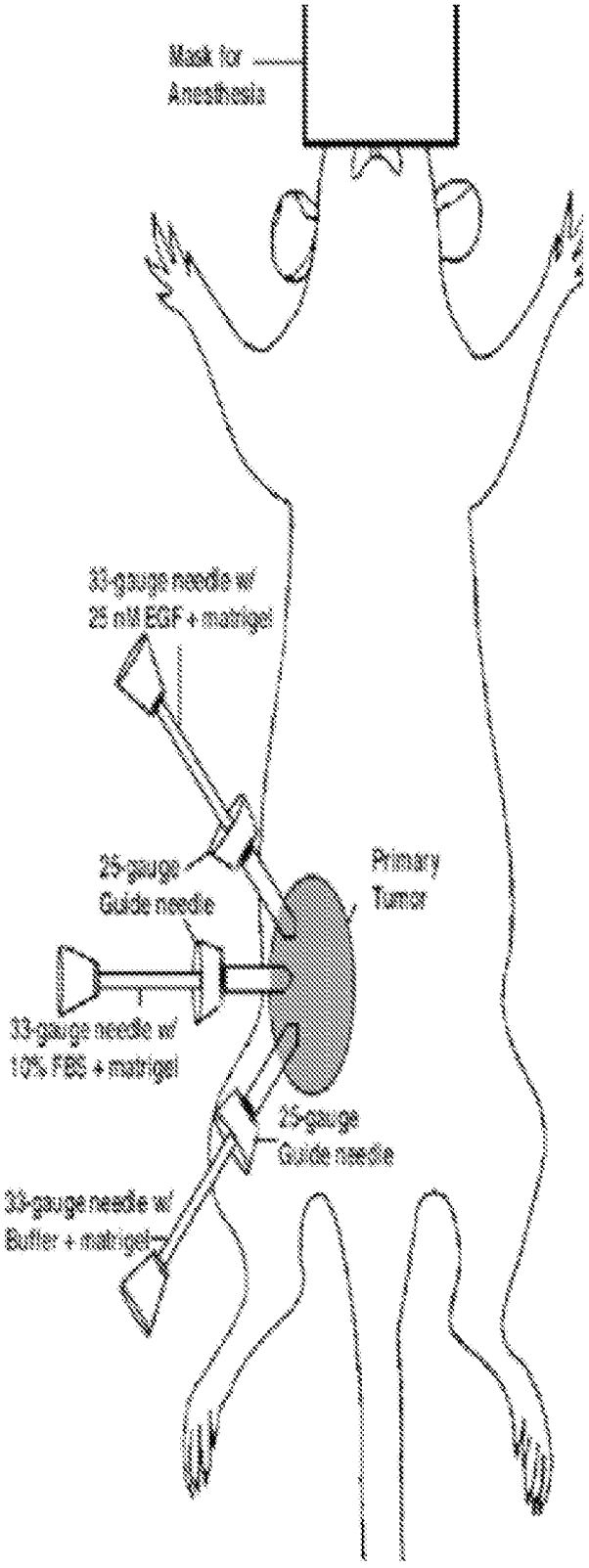
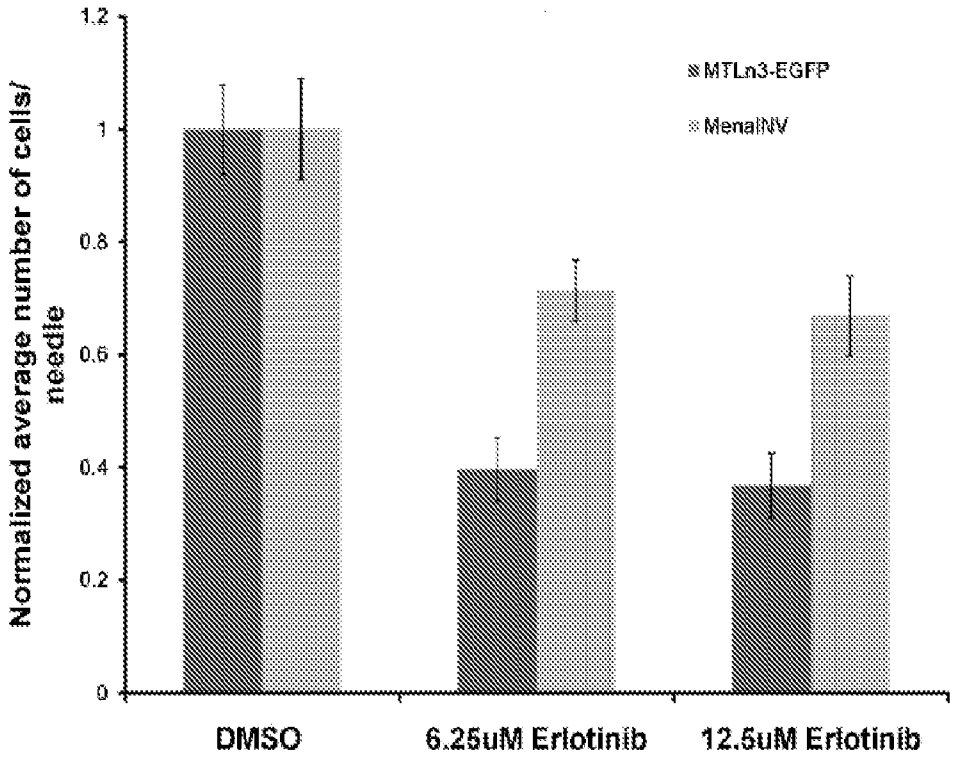

Mask for
Anesthesia
33-gauge needle w/
25 mM EGF + matrigel
25-gauge
Guide needle
Primary
Tumor
33-gauge needle w/
10% FBS + matrigel
25-gauge
Guide needle
33-gauge needle w/
Buffer + matrigel

FIGURE 2B

A



B

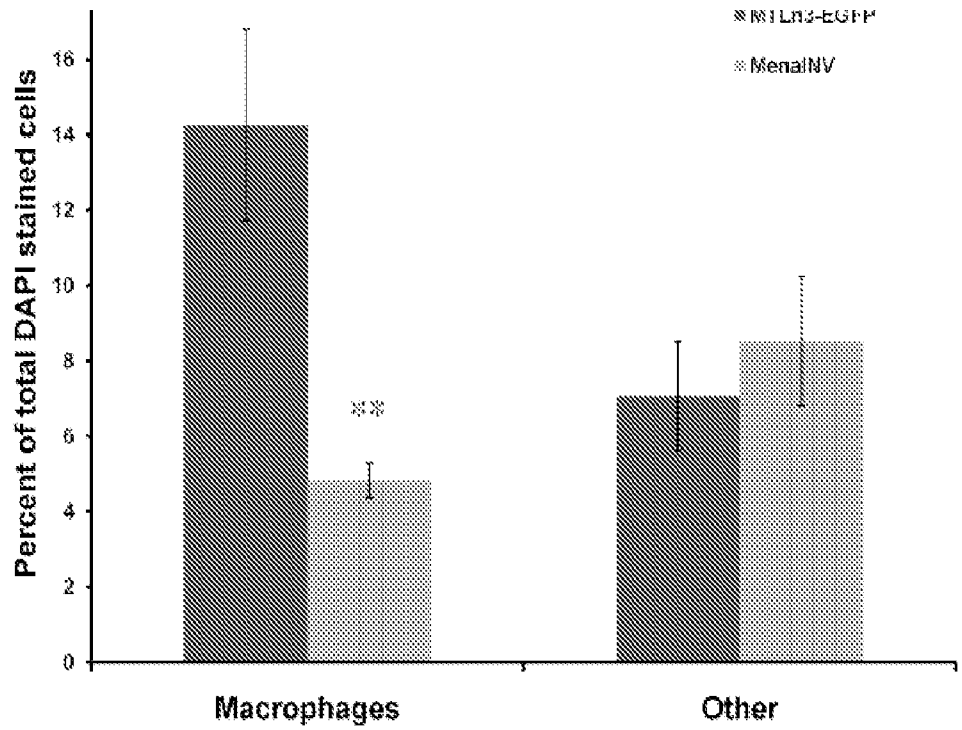


FIGURE 3A-3B

C

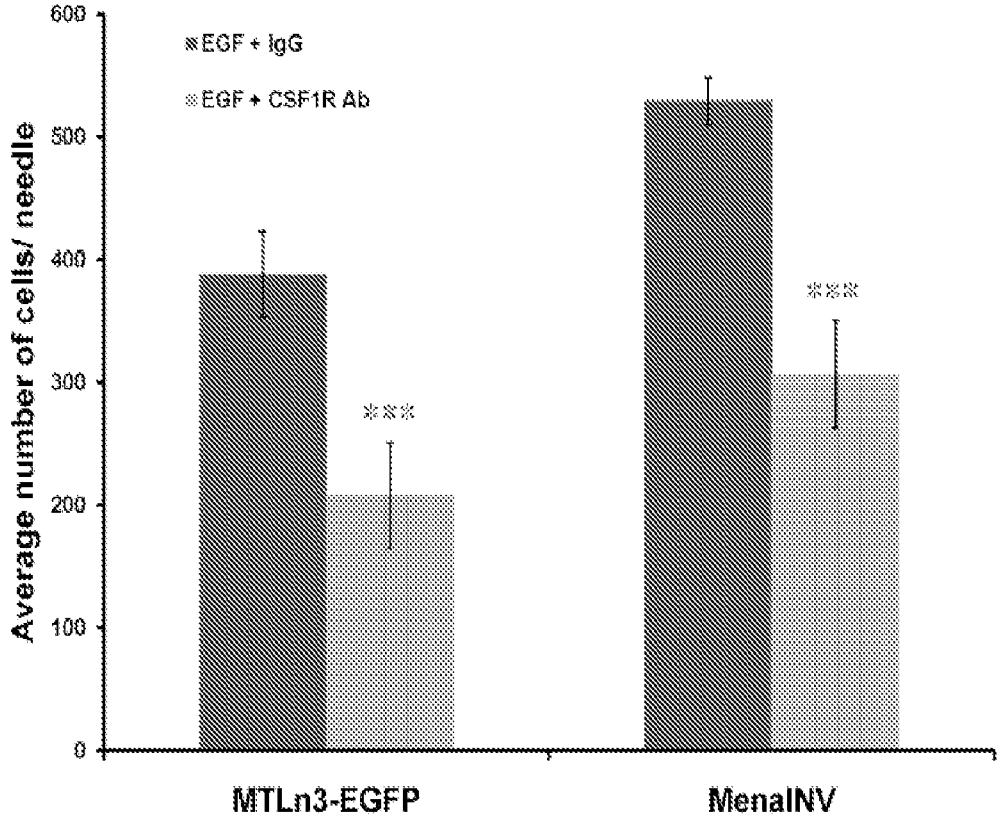
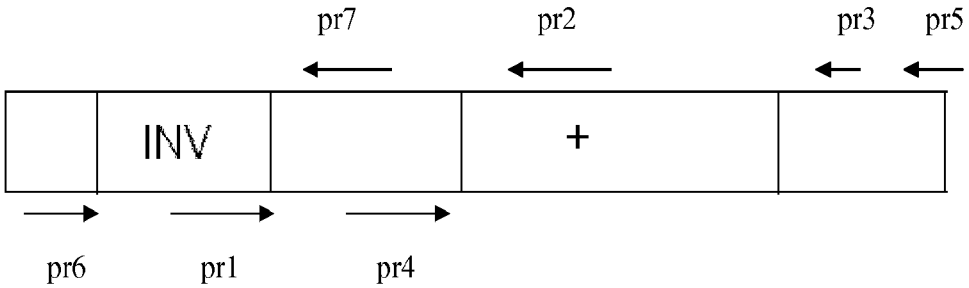

600
500
400
300
200
100
0
Average number of cells/ needle
EGF + IgG
EGF + CSF1R Ab
***
***
MTLn3-EGFP
MenaINV

FIGURE 3C

A



B

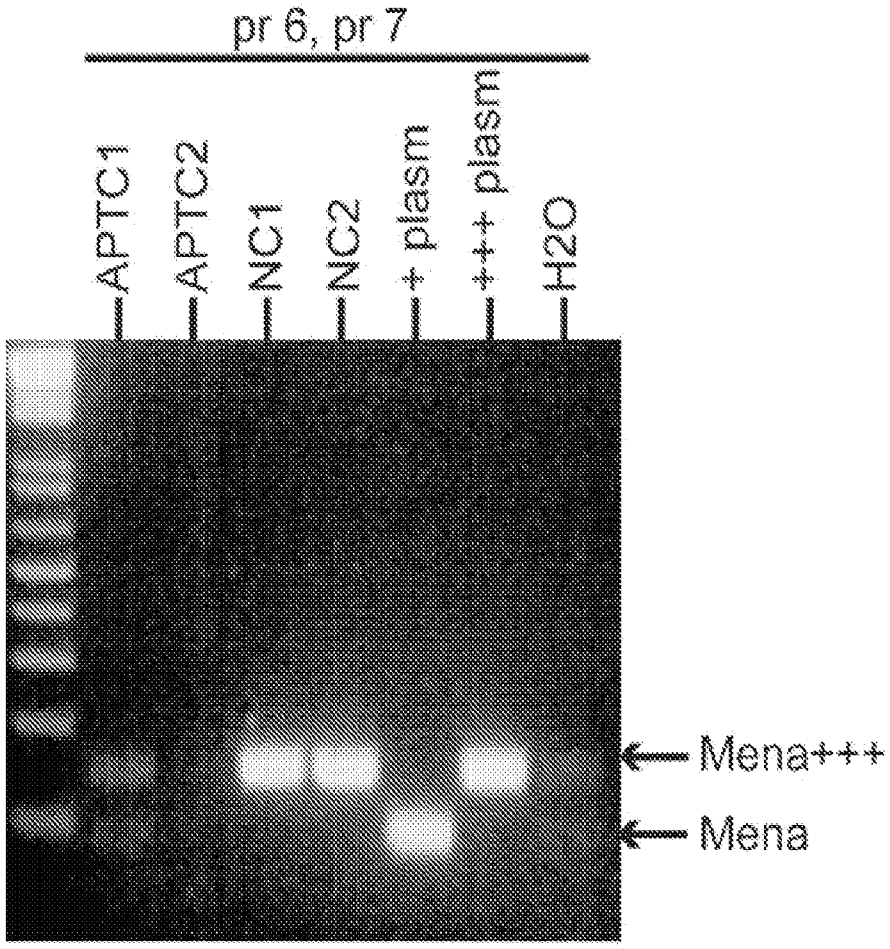
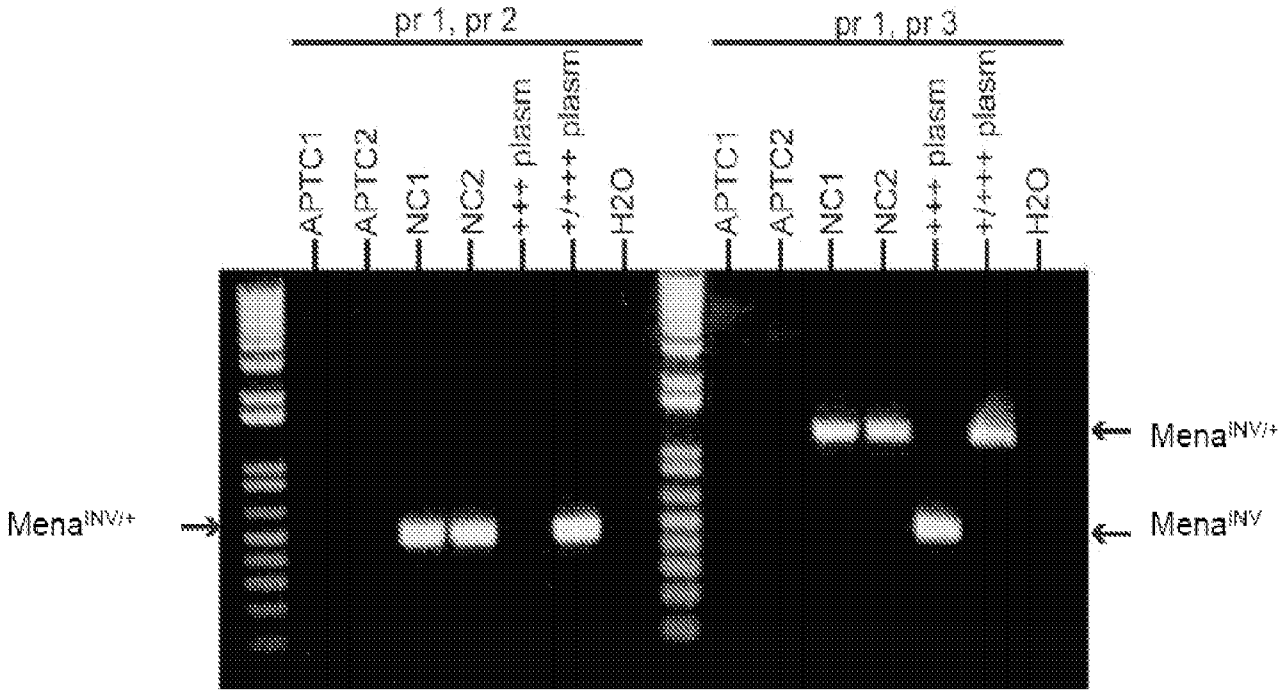


FIGURE 4A-4B

C



D

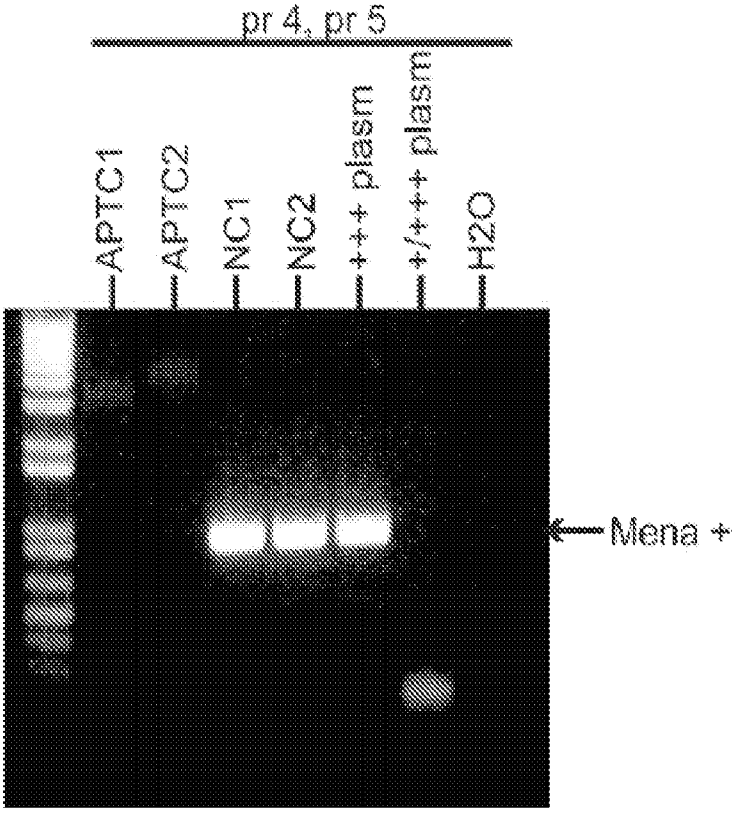


FIGURE 4C-4D

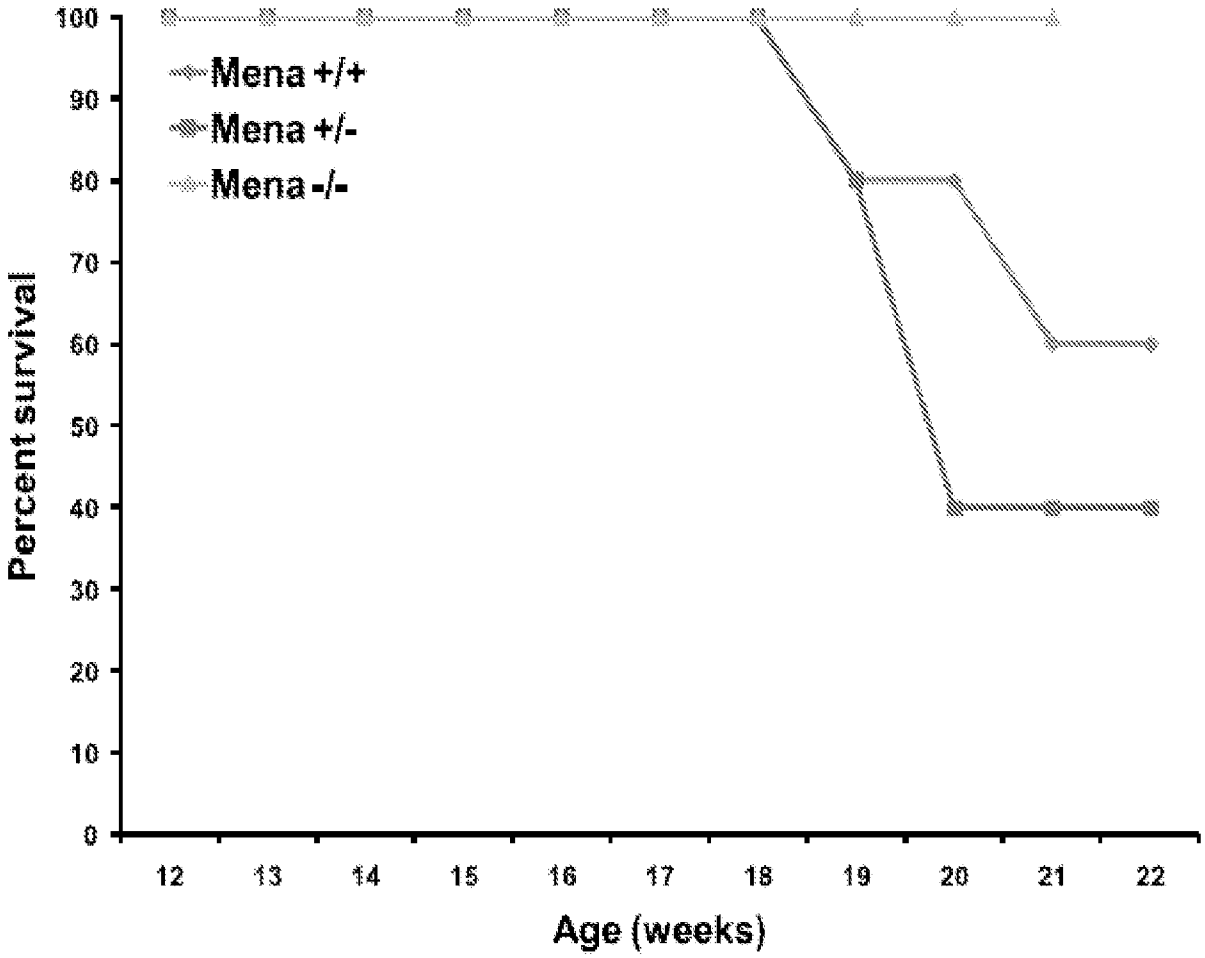

Mena +/+
Mena +/-
Mena -/-
Percent survival
100
90
80
70
60
50
40
30
20
10
0
12
13
14
15
16
17
18
19
20
21
22
Age (weeks)

FIGURE 5

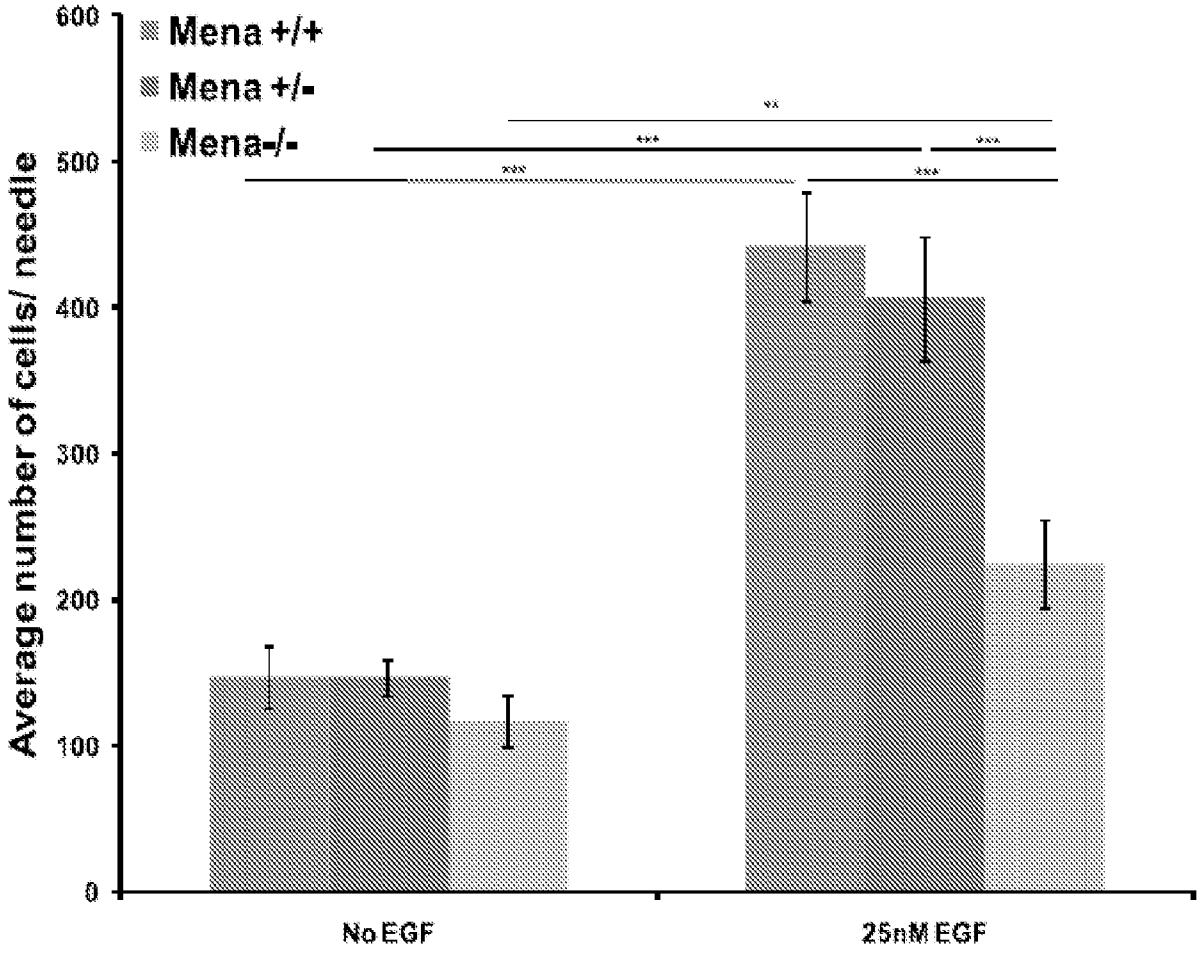

Mena +/+
Mena +/-
Mena-/-
Average number of cells/ needle
600
500
400
300
200
100
0
No EGF
25nM EGF

FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/00095

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - G01N 33/53 (2011.01)
USPC - 435/7.1
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)
USPC -- 435/7.1, 424/135.1, 435/6, 530/330

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST -- PGPB,USPT,USOC,EPAB,JPAB; Dialog Classic Files -- 654, 652, 349, 35, 65, 155; USPTO Web Page; PCT Patentscope; Google Scholar; Search terms -- Mena+ inhibitor, compound screen, EGF gradient, cell protrusion assay, metastasis inhibitor, epithelial cancer, breast cancer, in vivo, prognosis, cell invasion assay, treatement, oral, parenteral, p

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/097466 A2 (CONDEELIS et al.) 14 August 2008 (14.08.2008) para [0006], [0008]-[0012], [0014], [0027]-[0029], [0039], [0040], [0045], [0046], Fig 6	1-3, 10-16, 20, 24, 25, 27-39
Y	US 2003/004514 A1 (SHIMKETS et al.) 20 March 2003 (20.030.2003) para [0119], [0120], [0124]	1-3, 10-16, 20, 24, 25, 27-39
Y	US 2008/01388805 A1 (CONDEELIS) 12 June 2008 (12.06.2008) para [0078], [0088], [0089], [0097], [0103], [0105], [0142], [0152], [0153], [0158], [0167], [0174], [0176], [0182], [0189], Fig 10	1-3, 13-15
Y	US 2004/0235956 A1 (QUAY) 25 November 2004 (25.11.2004) para [0017], [0042], [0045], [0047]	16, 24, 25, 27-29
Y	PHILIPPAR, U. et al. A Mena Invasion Isoform Potentiates EGF-Induced Carcinoma Cell Invasion and Metastasis. Developmental Cell, December 2008, Vol. 15, No. 6, pages 813-828; pg 815, para 1; pg 822, para 3	31, 33, 35, 36/(31,33,35), 37/(31,33,35)

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"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)
"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
"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 March 2011 (09.03.2011)	04 APR 2011

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 11/00095

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of 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. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims 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. ☒ Claims Nos.: 4-9, 17-19, 21-23 and 26
because they are dependent 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 claims 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 claims 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.

专利名称(译)	用于确定靶向甲萘亚胺同种型的药剂的方法及其用于诊断和治疗转移性肿瘤的用途		
公开(公告)号	EP2529225A4	公开(公告)日	2013-08-14
申请号	EP2011737389	申请日	2011-01-19
[标]申请(专利权)人(译)	爱因斯坦医学院的Yeshiva UNIV医学院 麻省理工学院		
申请(专利权)人(译)	爱因斯坦医学院叶史瓦大学医学院 麻省理工学院		
当前申请(专利权)人(译)	爱因斯坦医学院叶史瓦大学医学院 麻省理工学院		
[标]发明人	GERTLER FRANK CONDEELIS JOHN		
发明人	GERTLER, FRANK CONDEELIS, JOHN		
IPC分类号	G01N33/53		
CPC分类号	G01N33/5011 G01N33/5088 G01N2500/04 G01N2800/56		
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摘要(译)

本发明涉及确定抑制Mena +或MenaINV / +的药剂的方法，以及结合和/或抑制Mena +或MenaINV / +的药剂用于诊断和治疗转移性肿瘤的用途。