REYES & REYES
A B O G A D O S

Señor

SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

E. S. D.

REF: **Expediente 11-014-579**

TÍTULO SOLICITADO: **"AGENTES Y ANTAGONISTAS FIJADORES DE NOTCH Y METODOS PARA EL USO DE LOS MISMOS"**

PUBLICACIÓN: **Gaceta 661 de 01 de Febrero de 2013, Nº de publicación 2253.**

PRIORIDAD:

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US 61/112701	**07/11/2008**
US 61/112699	**07/11/2008**

SOLICITANTE: **ONCOMED PHARMACEUTICALS INC**

APODERADO: **CARLOS REINALDO OLARTE GARCIA**

TRÁMITE: **SUSTENTACION DE OPOSICIÓN**

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUÍMICAS S.A.**, dentro del trámite de la referencia, en ejercicio de lo dispuesto por el artículo 42 inciso 2º de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada 2 de mayo de 2103, en los siguientes términos:

I. RATIFICACIÓN

Comedidamente me permito insistir en que la solicitud de la referencia, relacionada con composiciones que comprenden un agente que se une a un receptor Notch humano y métodos de uso de las composiciones para el

tratamiento del cáncer y otras enfermedades, incumple los requisitos materiales de patentabilidad para acceder al privilegio solicitado:

- La solicitud carece de claridad y soporte en la descripción, por cuanto ha sido definida por los ligandos a los cuales se unen los anticuerpos y no por las características técnicas estructurales para definirlos; de esta forma, la descripción no reúne datos e información necesarios que permitan dar soporte a las reivindicaciones presentadas.
- La materia reclamada por las reivindicaciones, se refiere a un anticuerpo definido porque se une a una molécula diana específica y no mediante la estructura técnica del anticuerpo tal como su secuencia.
- Las reivindicaciones 6, 18 y 25, caracterizan el compuesto que se une a un receptor Notch humano mediante la constante de disociación, la cual es una variable que mide el comportamiento del anticuerpo como tal y no constituye una característica técnica que permita definir la materia, por tal razón, le resta claridad y concisión a la solicitud.
- La reivindicación 30 reclama dos anticuerpos que no tienen relación entre si, en consecuencia, le resta coherencia y consistencia al alcance del mismo.
- La reivindicación 20 (independiente) por la forma en que ha sido reclamado el anticuerpo se entiende que el mismo ha sido extraído de la naturaleza, por lo tanto, se debe considerar como una excepción de patentabilidad tal como lo menciona el artículo 15 literal b) de la Decisión 486, pues contendría materia aislada de la naturaleza.
- Las reivindicaciones 59 a 89 que se refieren a un método para tratar a un paciente no son patentables ya que violan el artículo 20 literal d) de la Decisión 486.
- La novedad de la solicitud se encuentra afectada según lo revelado en la publicación americana US2008/0131434 ***"Compositions and methods for diagnosing and treating cáncer"***, (junio 5 de 2008), que divulga un anticuerpo capaz de unirse al receptor NOTCH2, y la producción de los mismos, tal como se reclama en la presente solicitud.

  Adicionalmente, el documento US2008/0131434, ya revelaba un anticuerpo asilado unido específicamente a una región no ligando del dominio extracelular de Notch, de la misma manera que lo hace la solicitud en trámite en su reivindicación 29.

II. COMPLEMENTO DE INFORMACION

Adicional a lo expresado anteriormente, me permito aportar otros argumentos que demuestran una vez más la ausencia de nivel inventivo de la solicitud en trámite, veamos:

- La solicitud presenta un elevado número de reivindicaciones, algunas de las cuales son reivindicaciones independientes que no cumplen con los requisitos de unidad de invención y/o describen distintos objetos inventivos que no cumplen obviamente con los requisitos de patentabilidad.

- Tal como se ha discutido en el escrito de oposición y en éste, las reivindicaciones carecen de claridad, concisión y soporte en la descripción y adicionalmente, es del caso mencionar que las mismas han sido anticipadas por el estado de la técnica como se analiza a continuación:

1. *Un anticuerpo aislado el cual se fija de manera específica a una región no fijadora de ligandos de un dominio extracelular de la Notch2 humana, en donde el anticuerpo se fija de manera específica a la repetición 10 EGF de la Notch2 humana.* *(Reivindicación 1 de la CO 11-014-579)*

Se observa que las características técnicas contempladas en la reivindicación 1, **han sido divulgadas en el estado del arte** tal como lo revela la publicación americana citada US2008/0131434. Ejemplo de ello es mostrado en la reivindicación 3 de dicho documento:

> 3. The antibody of claim 1, wherein the antibody specifically binds to a non-ligand binding region of the extracellular domain of NOTCH2.

De igual manera, en ejemplos preferidos de la anterioridad en mención, los anticuerpos se fijan de manera concreta a la repetición 10 EGF de la Notch 2 humana, tal como se ve a continuación:

> 10. The antibody of claim 1, wherein the non-ligand binding region comprises EGF repeats 1-10.

Así, dicho anticuerpo se une específicamente a una región que comprende las repeticiones EGF 1-10 del dominio extracelular del receptor Notch humano e inhibe el crecimiento de células tumorales.

Por su parte, el párrafo 0097 de la anterioridad en mención, demuestra que no hay diferencia con lo reclamado en la solicitud en trámite en su reivindicación 1:

> **[0097]** In certain embodiments the present invention provides an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 1-10 of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells. In certain embodiments the present invention provides an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 13-36 of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells. Certain embodiments provide an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 4 of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells. Certain embodiments provide an antibody that specifically binds to a non-ligand binding region comprising EGF repeats 13 of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells.

Así pues, queda demostrado que todas las características de la reivindicación 1 han sido anticipadas por la anterioridad en mención y, en consecuencia, las reivindicaciones dependientes por no aportar una diferencia sustancial, también son afectadas por dicho documento.

Para que quede más claro lo anterior, llamamos la atención de lo revelado en la reivindicación 2 y 3 de la solicitud en estudio:

2. *El anticuerpo de la reivindicación 1, el cual es un antagonista de la Notch 2 humana.*

(Solicitud colombiana en trámite)

> **3.** The antibody of claim **1**, wherein the antibody specifically binds to a non-ligand binding region of the extracellular domain of NOTCH2.

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Como se observa, la reivindicación 2 ha sido anticipada por la materia divulgada en la anterioridad tantas veces mencionada, en especial, en su reivindicación 3.

Por su parte, la reivindicación 3 de la solicitud en trámite que reclama como parte de su invención la fijación de un ligando con la Notch2 humana, es divulgada en el párrafo 0021 de la anterioridad así:

3. *El anticuerpo de la reivindicación 2, el cual inhibe la fijación de un ligando con una Notch2 humana.*

(Solicitud colombiana en trámite)

> **[0021]** The invention further provides a method of treating cancer in a human comprising administering to the human therapeutically effective amounts of (a) a first antibody which binds a Notch receptor and inhibits growth of cancer stem cells which overexpress Notch receptors; and (b) a second antibody which binds a Notch receptor and blocks ligand activation of a Notch receptor.
>
> *US 2008/0131434*

Por otro lado, la reivindicación independiente 20 que demuestra la evidente falta de unidad de invención reclama:

> *20. Un anticuerpo aislado el cual se fija de manera específica a una región no fijadora de ligandos de un dominio extracelular de la Notch3 humana, en donde el anticuerpo se fija de manera específica a la repetición 9 EGF de la Notch3 humana.*

En relación con dicha reivindicación independiente (20) que señala específicamente la repetición 9 EGF de la Notch 3 humana, el documento de anterioridad tantas veces citado, demuestra la unión a Notch 3 humana (párrafo 0094), veamos:

[0094] The present invention further identifies molecules (e.g. antibodies) that specifically bind to a non-ligand binding region of the extracellular domain of a human NOTCH receptor and inhibit tumor growth in vivo. The ligand binding region of Notch, which is necessary and sufficient for ligand binding, has been identified as EGF repeats 11 and 12, suggesting this region of the Notch receptor is important in Notch signaling and turmorigenesis (Rebay et al., 1991, Cell 67:687; Lei et al., 2003, Dev. 130:6411; Hambleton et al., 2004, Structure 12:2173). Unexpectedly and for the first time, antibodies that bind outside the ligand binding domain of the extracellular domain of human Notch receptor were found to inhibit tumor cell growth in vivo. One such antibody to an epitope within EGF repeat 4 of NOTCH1 inhibited tumor cell growth in an animal model. These results suggest that antibodies that bind outside the ligand binding domain of the extracellular domain of one or more of the human Notch receptors—NOTCH1, NOTCH2, NOTCH3, and NOTCH4—have value as potential cancer therapeutics.

En adición a lo expuesto, el anticuerpo divulgado previamente se une más específicamente a la repetición 9 del Notch 3 humano, como se observó en el texto extraído de la anterioridad del párrafo 0097.

Ahora bien, para demostrar una vez más la falta de unidad de invención, se observa que uno de los grupos inventivos definido por la reivindicación independiente 82 revela materia que se refiere tanto a una excepción a la patentabilidad como a un objeto inventivo diferente al los ya analizados:

> *82. Un método de inhibir la angiogénesis en un sujeto, el cual comprende administrar al sujeto una cantidad efectiva de la Notch2 y/o Notch 3 humanas.*

Las características técnicas esenciales en este caso, son anticipadas por la publicación americana US 2006/0030694 de Kitajewski et al., el cual divulga un método de angiogénesis en un sujeto que comprende la administración de una composición de materia que comprende el dominio extracelular del receptor Notch para inhibir la angiogénesis en un sujeto:

> **[0020]** This invention also provides a method for inhibiting angiogenesis in a subject comprising administering to the subject an effective amount of a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety, so as to thereby inhibit angiogenesis in the subject.

Adicionalmente, la anterioridad ya había divulgado la administración de una cantidad efectiva del antagonista del Notch 2 humano a un sujeto:

> **[0113]** In a second embodiment of the above methods, the Notch receptor protein is Notch2 receptor protein. In one embodiment, the Notch2 receptor protein is human Notch2 receptor protein. In another embodiment, the half-life-increasing moiety is an Fc portion of an antibody. In another embodiment, the Fc portion of the antibody is the Fc portion of a human antibody. In a further embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

Por lo tanto, los anticuerpos, los métodos y demás objetos reclamados en la presente solicitud, ya habían sido enseñados en el estado del arte; adicionalmente, los anticuerpos reclamados al no ser nuevos, no demuestran un efecto inesperado o una mejora sorprendentes sobre lo mencionado en las anterioridades, ya que siguen actuando sobre los mismos receptores, con una constante de disociación que no describe un cambio en la efectividad de unión o estabilidad y producen el mismo efecto.

Así las cosas, y con fundamento en los argumentos expuestos a lo largo del presente escrito, respetuosamente reitero la solicitud para que su despacho se abstenga de conceder el privilegio de patente solicitado, y ordene en consecuencia el archivo del expediente respectivo.

III. PRUEBAS

- La publicación americana US2008/0131434 ***"Compositions and methods for diagnosing and treating cáncer"***, del 5 de junio de 2008. Portada, páginas 1, 2, 28, 42, y reivindicaciones.

- La publicación americana US 2006/0030694 ***"Notch-based fusión proteins and uses thereof"*** publicado por Kitajewski et al., el 9 de febrero de 2006. Portada, páginas 1, 2, 7.

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Lewicki et al.

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(54) **COMPOSITIONS AND METHODS FOR DIAGNOSING AND TREATING CANCER**

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(60) Provisional application No. 60/812,955, filed on Jun. 13, 2006, provisional application No. 60/879,336, filed on Jan. 9, 2007, provisional application No. 60/878,661, filed on Jan. 5, 2007.

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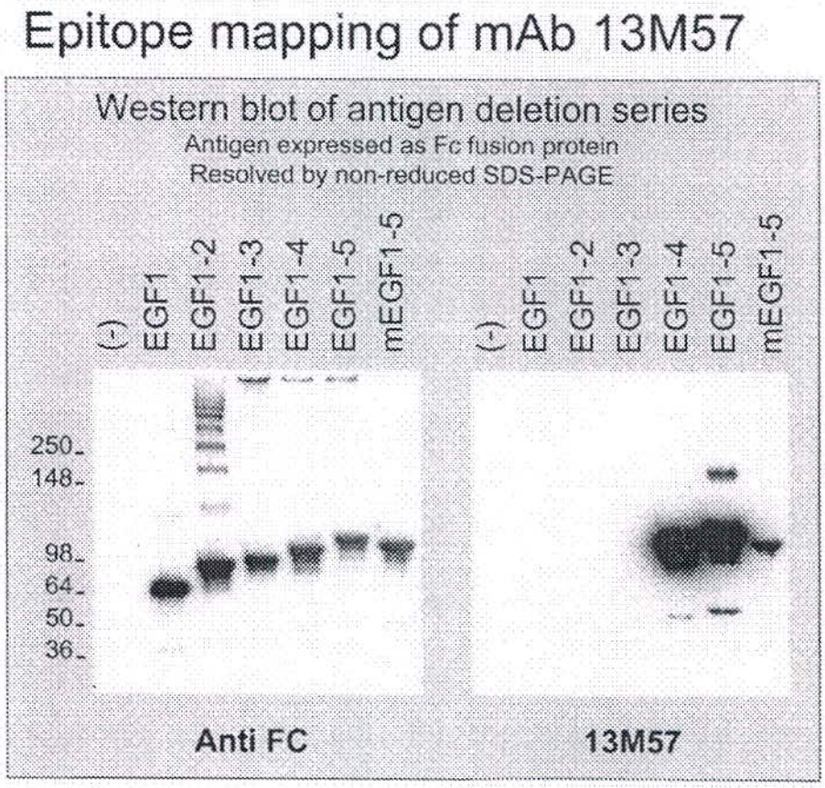
(57) **ABSTRACT**

The present invention relates to compositions and methods for characterizing, diagnosing, and treating cancer. In particular the invention provides the means and methods for the diagnosis, characterization, prognosis and treatment of cancer and specifically targeting cancer stem cells. The present invention provides an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells. The present invention further provides a method of treating cancer, the method comprising administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor protein and inhibits growth of tumor cells.



COMPOSITIONS AND METHODS FOR DIAGNOSING AND TREATING CANCER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Prov. Appl. No. 60/812,955, filed Jun. 13, 2006; U.S. Prov. Appl. No. 60/879,336, filed Jan. 9, 2007; and U.S. Prov. Appl. No. 60/878,661, filed Jan. 5, 2007; each of which are herein incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention relates to compositions and methods for characterizing, diagnosing, and treating cancer. In particular, the invention provides the means and methods for diagnosis, characterization, prognosis, and treatment of cancer and specifically targeting cancer stem cells. The present invention provides an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells. The present invention further provides a method of treating cancer, the method comprising administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor protein and inhibits growth of tumor cells.

[0004] 2. Background Art

[0005] Cancer is one of the leading causes of death in the developed world, resulting in over 500,000 deaths per year in the United States alone. Over one million people are diagnosed with cancer in the U.S. each year, and overall it is estimated that more than 1 in 3 people will develop some form of cancer during their lifetime. Though there are more than 200 different types of cancer, four of them—breast, lung, colorectal, and prostate—account for over half of all new cases (Jemal et al., *Cancer J. Clin.* 53:5-26 (2003)).

[0006] Breast cancer is the most common cancer in woman, with an estimate 12% of women at risk of developing the disease during their lifetime. Although mortality rates have decreased due to earlier detection and improved treatments, breast cancer remains a leading cause of death in middle-aged women. Furthermore, metastatic breast cancer is still an incurable disease. On presentation, most patients with metastatic breast cancer have only one or two organ systems affected, but as the disease progresses, multiple sites usually become involved. The most common sites of metastatic involvement are locoregional recurrences in the skin and soft tissues of the chest wall, as well as in axilla and supraclavicular areas. The most common site for distant metastasis is the bone (30 40% of distant metastasis), followed by the lungs and liver. And although only approximately 1-5% of women with newly diagnosed breast cancer have distant metastasis at the time of diagnosis, approximately 50% of patients with local disease eventually relapse with metastasis within five years. At present the median survival from the manifestation of distant metastases is about three years.

[0007] Current methods of diagnosing and staging breast cancer include the tumor-node-metastasis (TNM) system that relies on tumor size, tumor presence in lymph nodes, and the presence of distant metastases as described in the American Joint Committee on Cancer: AJCC Cancer Staging Manual. Philadelphia, Pa.: Lippincott-Raven Publishers, 5th ed., 1997, pp 171-180, and in Harris, J R: "Staging of breast carcinoma" in Harris, J. R., Hellman, S., Henderson, I. C., Kinne D. W. (eds.): Breast Diseases. Philadelphia, Lippincott, 1991. These parameters are used to provide a prognosis and select an appropriate therapy. The morphologic appearance of the tumor may also be assessed but because tumors with similar histopathologic appearance can exhibit significant clinical variability, this approach has serious limitations. Finally assays for cell surface markers can be used to divide certain tumor types into subclasses. For example, one factor considered in the prognosis and treatment of breast cancer is the presence of the estrogen receptor (ER) as ER-positive breast cancers typically respond more readily to hormonal therapies such as tamoxifen or aromatase inhibitors than ER-negative tumors. Yet these analyses, though useful, are only partially predictive of the clinical behavior of breast tumors, and there is much phenotypic diversity present in breast cancers that current diagnostic tools fail to detect and current therapies fail to treat.

[0008] Prostate cancer is the most common cancer in men in the developed world, representing an estimated 33% of all new cancer cases in the U.S., and is the second most frequent cause of death (Jemal et al., 2003, CA Cancer J. Clin. 53:5-26). Since the introduction of the prostate specific antigen (PSA) blood test, early detection of prostate cancer has dramatically improved survival rates, and the five year survival rate for patients with local and regional stage prostate cancers at the time of diagnosis is nearing 100%. Yet more than 50% of patients will eventually develop locally advanced or metastatic disease (Muthuramalingam et al., 2004, Clin. Oncol. 16:505-16).

[0009] Currently radical prostatectomy and radiation therapy provide curative treatment for the majority of localized prostate tumors. However, therapeutic options are very limited for advanced cases. For metastatic disease, androgen ablation with luteinising hormone-releasing hormone (LHRH) agonist alone or in combination with anti-androgens is the standard treatment. Yet despite maximal androgen blockage, the disease nearly always progresses with the majority developing androgen-independent disease. At present there is no uniformly accepted treatment for hormone refractory prostate cancer, and chemotherapeutic regimes are commonly used (Muthuramalingam et al., 2004, Clin. Oncol. 165:505-16; Trojan et al., 2005, Anticancer Res. 25:551-61).

[0010] Colorectal cancer is the third most common cancer and the fourth most frequent cause of cancer deaths worldwide (Weitz et al., 2005, Lancet 365:153-65). Approximately 5-10% of all colorectal cancers are hereditary with one of the main forms being familial adenomatous polyposis (FAP), an autosomal dominant disease in which about 80% of affected individuals contain a germline mutation in the adenomatous polyposis coli (APC) gene. Colorectal carcinoma has a tendency to invade locally by circumferential growth and elsewhere by lymphatic, hematogenous, transperitoneal, and perineural spread. The most common site of extralymphatic involvement is the liver, with the lungs the most frequently affected extra-abdominal organ. Other sites of hematogenous spread include the bones, kidneys, adrenal glands, and brain.

[0011] The current staging system for colorectal cancer is based on the degree of tumor penetration through the bowel wall and the presence or absence of nodal involvement. This staging system is defined by three major Duke's classifications: Duke's A disease is confined to submucosa layers of colon or rectum; Duke's B disease has tumors that invade

through the muscularis propria and may penetrate the wall of the colon or rectum; and Duke's C disease includes any degree of bowel wall invasion with regional lymph node metastasis. While surgical resection is highly effective for early stage colorectal cancers, providing cure rates of 95% in Duke's A patients, the rate is reduced to 75% in Duke's B patients and the presence of positive lymph node in Duke's C disease predicts a 60% likelihood of recurrence within five years. Treatment of Duke's C patients with a post surgical course of chemotherapy reduces the recurrence rate to 40%-50%, and is now the standard of care for these patients.

[0012] Lung cancer is the most common cancer worldwide, the third most commonly diagnosed cancer in the United States, and by far the most frequent cause of cancer deaths (Spiro et al., 2002, Am. J. Respir. Crit. Care Med. 166:1166-96; Jemal et al., 2003, CA Cancer J. Clin. 53:5-26). Cigarette smoking is believed responsible for an estimated 87% of all lung cancers, making it the most deadly preventable disease. Lung cancer is divided into two major types that account for over 90% of all lung cancers: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC accounts for 15-20% of cases and is characterized by its origin in large central airways and histological composition of sheets of small cells with little cytoplasm. SCLC is more aggressive than NSCLC, growing rapidly and metastasizing early and often. NSCLC accounts for 80-85% of all cases and is further divided into three major subtypes based on histology: adenocarcinoma, squamous cell carcinoma (epidermoid carcinoma), and large cell undifferentiated carcinoma.

[0013] Lung cancer typically presents late in its course, and thus has a median survival of only 6-12 months after diagnosis and an overall 5 year survival rate of only 5-10%. Although surgery offers the best chance of a cure, only a small fraction of lung cancer patients are eligible with the majority relying on chemotherapy and radiotherapy. Despite attempts to manipulate the timing and dose intensity of these therapies, survival rates have increased little over the last 15 years (Spiro et al., 2002, Am. J. Respir. Crit. Care Med. 166:1166-96).

[0014] Cancer arises from dysregulation of the mechanisms that control normal tissue development and maintenance, and increasingly stem cells are thought to play a central role (Bleachy et al., 2004, Nature 432:324). During normal animal development, cells of most or all tissues are derived from normal precursors, called stem cells (Morrison et al., 1997, Cell 88:287-98; Morrison et al., 1997, Curr. Opin. Immunol. 9:216-21; Morrison et al., 1995, Annu. Rev. Cell. Dev. Biol. 11:35-71). Stem cells are cell that: (1) have extensive proliferative capacity; 2) are capable of asymmetric cell division to generate one or more kinds of progeny with reduced proliferative and/or developmental potential; and (3) are capable of symmetric cell divisions for self-renewal or self-maintenance. The best-known example of adult cell renewal by the differentiation of stem cells is the hematopoietic system where developmentally immature precursors (hematopoietic stem and progenitor cells) respond to molecular signals to form the varied blood and lymphoid cell types. Other cells, including cells of the gut, breast ductal system, and skin are constantly replenished from a small population of stem cells in each tissue, and recent studies suggest that most other adult tissues also harbor stem cells, including the brain.

[0015] Solid tumors are composed of heterogeneous cell populations. For example, breast cancers are a mixture of cancer cells and normal cells, including mesenchymal (stromal) cells, inflammatory cells, and endothelial cells. Classic models of cancer hold that phenotypically distinct cancer cell populations all have the capacity to proliferate and give rise to a new tumor. In the classical model, tumor cell heterogeneity results from environmental factors as well as ongoing mutations within cancer cells resulting in a diverse population of tumorigenic cells. This model rests on the idea that all populations of tumor cells would have some degree of tumorigenic potential. (Pandis et al., 1998, Genes, Chromosomes & Cancer 12:122-129; Kuukasjrvi et al., 1997, Cancer Res. 57:1597-1604; Bonsing et al., 1993, Cancer 71:382-391; Bonsing et al., 2000, Genes Chromosomes & Cancer 82: 173-183; Beerman H et al., 1991, Cytometry. 12:147-54; Aubele M & Werner M, 1999, Analyt. Cell. Path. 19:53; Shen L et al., 2000, Cancer Res. 60:3884).

[0016] An alternative model for the observed solid tumor cell heterogeneity is that solid tumors result from a "solid tumor stem cell" (or "cancer stem cell" from a solid tumor) that subsequently undergoes chaotic development through both symmetric and asymmetric rounds of cell divisions. In this stem cell model, solid tumors contain a distinct and limited (possibly even rare) subset of cells that share the properties of normal "stem cells", in that they extensively proliferate and efficiently give rise both to additional solid tumor stem cells (self-renewal) and to the majority of tumor cells of a solid tumor that lack tumorigenic potential. Indeed, mutations within a long-lived stem cell population may initiate the formation of cancer stem cells that underlie the growth and maintenance of tumors and whose presence contributes to the failure of current therapeutic approaches.

[0017] The stem cell nature of cancer was first revealed in the blood cancer, acute myeloid leukemia (AML) (Lapidot et al., 1994, Nature 17:645-8). More recently it has been demonstrated that malignant human breast tumors similarly harbor a small, distinct population of cancer stem cells enriched for the ability to form tumors in immunodeficient mice. An ESA+, CD44+, CD24−/low, Lin− cell population was found to be 50-fold enriched for tumorigenic cells compared to unfractionated tumor cells (Al-Hajj et al., 2003, PNAS 100: 3983-8). The ability to prospectively isolate the tumorigenic cancer cells has permitted investigation of critical biological pathways that underlie tumorigenicity in these cells, and thus promises the development of better diagnostic assays and therapeutics for cancer patients. It is toward this purpose that this invention is directed.

BRIEF SUMMARY OF THE INVENTION

[0018] The present invention provides a method of treating cancer, wherein the cancer comprises cancer stem cells, comprising administering to the subject a therapeutically effective amount of an antibody which binds a cancer stem cell marker. In a more particular aspect, the present invention provides a method of treating cancer, wherein the cancer comprises stem cells expressing one or more Notch receptor family members, comprising administering to the subject a therapeutically effective amount of an antibody that binds those Notch receptor family members or the ligands to those Notch receptors. The present invention for the first time identifies antibodies that bind to the non-ligand binding domain of the extracellular domain of a human NOTCH receptor as therapeutically effective against cancer. Thus in certain embodiments the present invention provides an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells.

NOTCH receptor are tested for the ability to inhibit tumor growth in vivo. In certain embodiments, molecules that specifically bind a non-ligand binding region of the extracellular domain of a human NOTCH receptor are injected into an animal xenograft model and the growth of tumors in animals treated with molecules that specifically bind to non-ligand binding region of the extracellular domain of a human NOTCH receptor is determined and compared to animals treated with a non-binding control molecule.

EXAMPLES

Example 1

Production of Antibodies

Antigen Production

[0229] Antibodies were generated against the non-ligand binding region of NOTCH1 and NOTCH2.

[0230] In certain embodiments, recombinant polypeptide fragments of the human NOTCH1 extracellular domain were generated as antigens for antibody production. Standard recombinant DNA technology was used to isolate polynucleotides encoding amino acids 1-220 of NOTCH1 (SEQ ID NO: 1) and amino acids 1427-1563 of NOTCH1 (SEQ ID NO: 18). These polynucleotides were separately ligated in-frame N-terminal to a human Fc and histidine-tag and cloned into a transfer plasmid vector for baculovirus mediated expression in insect cells. Standard transfection, infection, and cell culture protocols were used to produce recombinant insect cells expressing the corresponding NOTCH1 polypeptides corresponding to EGF repeats 1-5 (SEQ ID NO: 2) and EGF repeats 10-15 (SEQ ID NO: 19) (O'Reilley et al., Baculovirus expression vectors: A Laboratory Manual, Oxford: Oxford University Press (1994)).

[0231] Cleavage of the endogenous NOTCH1 signal sequence was approximated using cleavage prediction software SignalP 3.0 at between amino acid 18 and 19, however the actual in vivo cleavage point can differ by a couple of amino acids either direction. Thus NOTCH1 antigen protein corresponding to EGF repeats 1-5 comprises approximately amino acid 19 through amino acid 220 (SEQ ID NO: 3). Antigen protein was purified from insect cell lysates using protein A and Ni++-chelate affinity chromatography. Purified antigen protein was dialyzed against PBS (pH=7), concentrated to approximately 1 mg/ml, and sterile filtered in preparation for immunization.

[0232] In certain embodiments, recombinant polypeptide fragments of the human NOTCH2 extracellular domain were generated as antigens for antibody production Standard recombinant DNA technology was used to isolate a polynucleotide encoding amino acids 1-493 of Notch2 (SEQ ID NO: 1). This polynucleotide was ligated in-frame N-terminal to either a human Fc-tag or histidine-tag and cloned into a transfer plasmid vector for baculovirus mediated expression in insect cells. Standard transfection, infection, and cell culture protocols were used to produce recombinant insect cells expressing the corresponding Notch2 polypeptide (O'Reilley et al., *Baculovirus expression vectors: A Laboratory Manual*, Oxford: Oxford University Press (1994)).

[0233] Cleavage of the endogenous signal sequence of human Notch2 was approximated using cleavage prediction software SignalP 3.0, however the actual in vivo cleavage point can differ by a couple of amino acids either direction. The predicated cleavage of Notch2 is between amino acids 1 and 26, thus Notch2 antigen protein comprises approximately amino acid 27 through amino acid 493. Antigen protein was purified from insect cell conditioned medium using Protein A and Ni^{++}-chelate affinity chromatography. Purified antigen protein was then dialyzed against PBS (pH=7), concentrated to approximately 1 mg/ml, and sterile filtered in preparation for immunization.

Immunization

[0234] Mice (n=3) were immunized with purified NOTCH1 or NOTCH2 antigen protein (Antibody Solutions; Mountain View, Calif.) using standard techniques. Blood from individual mice was screened approximately 70 days after initial immunization for antigen recognition using ELISA and FACS analysis (described in detail below). The two animals with the highest antibody titers were selected for final antigen boost after which spleen cells were isolated for hybridoma production. Hybridoma cells were plated at 1 cell per well in 96 well plates, and the supernatant from each well screened by ELISA and FACS analysis against antigen protein. Several hybridomas with high antibody titer were selected and scaled up in static flask culture. Antibodies were purified from the hybridoma supernatant using protein A or protein G agarose chromatography and antibodies were tested by FACS as described below. Several anti-NOTCH1 antibodies were isolated including 13M57 (also referred to as 13M30) (ATCC deposit no. _____; deposited _____) from animals immunized with NOTCH1 antigen corresponding to EGF repeats 1-5 and 31M80 (ATCC deposit no. _____; deposited _____), 31M103 (ATCC deposit no. _____; deposited _____), 31M106 (ATCC deposit no. _____; deposited _____), and 31M108 (ATCC deposit no. _____; deposited _____) from animals immunized with NOTCH1 antigen corresponding to EGF repeats 10-15. The nucleotide and predicted protein sequences of both the heavy chain (SEQ ID NO: 4-5) and light chain (SEQ ID NO: 6-7) of antibody 13M57 were determined. A NOTCH2 antibody, 59M07 (ATCC deposit no. _____; deposited _____), was generated that specifically binds to EGF repeat 2.

Epitope Mapping

[0235] To identify antibodies that recognize specific non-ligand binding regions of the NOTCH receptor extracellular domains, epitope mapping was preformed. In certain embodiments, mammalian expression plasmid vectors comprising a CMV promoter upstream of polynucleotides that encode fragments of the extracellular NOTCH1 domain as Fc fusion proteins were generated using standard recombinant DNA technology. These fusion proteins included a series of NOTCH1 fragments containing a nested series of deletions of EGF domains 1-5 or 10-15. Recombinant proteins were then expressed in cultured mammalian cells by transient transfection. Twenty-four to 48 hours following transfection, cells were harvested and cell lysate protein separated on non-reducing SDS-PAGE acrylamide gels for Western blotting using antibodies from mice immunized with NOTCH1 antigen. As shown in FIGS. 1A and B monoclonal antibody 13M57 recognized an epitope contained within EGF repeat 4 (SEQ ID NO: 8) with a K_D of 0.2 nM. Monoclonal antibody 31M80 recognized an epitope within EGF repeat 13 (FIG. 1C). Antibodies 31M103 (K_D=0.2 nM), 31M106 (K_D=3 nM),

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Thr Pro Cys Lys Asn Gly Ala Lys Cys Leu Asp Gly Pro Asn Thr Tyr
                165                 170                 175

Thr Cys Val Cys Thr Glu Gly Tyr Thr Gly Thr His Cys Glu Val Asp
            180                 185                 190

Ile Asp Glu Cys Asp Pro Asp Pro Cys His Tyr Gly Ser Cys Lys Asp
        195                 200                 205

Gly Val Ala Thr Phe Thr Cys Leu Cys Arg Pro Gly Tyr Thr Gly His
    210                 215                 220

His Cys Glu
225
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1. An isolated antibody that specifically binds to a non-ligand binding region of an extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells.

2. The isolated antibody of claim **1**, wherein the antibody specifically binds to a non-ligand binding region of the extracellular domain of NOTCH1.

3. The antibody of claim **1**, wherein the antibody specifically binds to a non-ligand binding region of the extracellular domain of NOTCH2.

4. The antibody of claim **1**, wherein the antibody specifically binds to a non-ligand binding region of the extracellular domain of at least two Notch receptor family members.

5. The antibody of claim **1**, wherein the antibody is a monoclonal antibody.

6. The antibody of claim **5**, wherein the antibody is a chimeric antibody.

7. The antibody of claim **5**, wherein the antibody is a humanized antibody.

8. The antibody of claim **5**, wherein the antibody is a human antibody.

9. A hybridoma producing the antibody of claim **5**.

10. The antibody of claim **1**, wherein the non-ligand binding region comprises EGF repeats 1-10.

11. The antibody of claim **10**, wherein the non-ligand binding region comprises EGF repeat 4.

12. The antibody of claim **11**, wherein the antibody is 13M57.

13. The antibody of claim **10**, wherein the non-ligand binding region comprises EGF repeat 2.

14. The antibody of claim **13**, wherein the antibody is 59M07.

15. The antibody of claim **1**, wherein the non-ligand binding region comprises EGF repeats 13-36.

16. The antibody of claim **15**, wherein the non-ligand binding region comprises EGF repeat 13.

17. The antibody of claim **16**, wherein the antibody is selected from the group consisting of 31 M80, 31μM103, 31M106, and 31M108.

18. An isolated antibody that competes with antibody 13M57 for specific binding to a non-ligand binding region of an extracellular domain of human NOTCH1, wherein the 13M57 antibody comprises: (a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS: 12, 13, and 14; and (b) a light chain variable region having CDR sequences set forth in SEQ ID NOS: 15, 16, and 17.

19. An isolated antibody that competes with an antibody selected from the group consisting of 31M80, 31M103, 31M106, and 31M108 for specific binding to a non-ligand binding region of an extracellular domain of human NOTCH1.

20. An isolated antibody that competes with antibody 59M07 for specific binding to a non-ligand binding region of an extracellular domain of human NOTCH2.

21. An isolated polypeptide that specifically binds to a non-ligand binding region of an extracellular domain of a human NOTCH receptor comprising: (a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS: 12, 13, and 14; and (b) a light chain variable region having CDR sequences set forth in SEQ ID NOS: 15, 16, and 17.

22. The isolated polypeptide of claim **21**, wherein the polypeptide comprises: (a) heavy chains set forth in SEQ ID NOS: 4 and 5; and (b) light chains set forth in SEQ ID NOS: 6 and 7.

23. A method of treating cancer, the method comprising administering a therapeutically effective amount of an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor protein and inhibits growth of tumor cells.

24-30. (canceled)

31. The method of claim **23**, wherein the non-ligand binding region comprises EGF repeats 1-10.

32. The method of claim **31**, wherein the non-ligand binding region comprises EGF repeat 4.

33. The method of claim **32**, wherein the antibody is 13M57.

34. The method of claim **31**, wherein the non-ligand binding region comprises EGF repeat 2.

35. The method of claim **34**, wherein the antibody is 59M07.

36. The method of claim **23**, wherein the non-ligand binding region comprises EGF repeats 13-36.

37. The method of claim **36**, wherein the non-ligand binding region comprises EGF repeat 13.

38. The method of claim **37**, wherein the antibody is selected from the group consisting of 31M80, 31M103, 31M106, and 31M108.

39. A method of treating cancer, the method comprising administering a therapeutically effective amount of an isolated polypeptide that specifically binds to a non-ligand binding region of an extracellular domain of a human NOTCH receptor comprising: (a) a heavy chain variable region having CDR sequences set forth in SEQ ID NOS: 12, 13, and 14; and

(b) a light chain variable region having CDR sequences set forth in SEQ ID NO: 15, 16, and 17.

40. The method of claim **39**, wherein the antibody comprises: (a) heavy chains set forth in SEQ ID NOS: 4 and 5; and (b) light chains set forth in SEQ ID NO: 6 and 7.

41. The method of claim **23**, wherein the antibody is conjugated to a cytotoxic moiety.

42-48. (canceled)

49. The method of claim **23**, wherein the antibody is administered with an additional antibody therapeutic.

50-54. (canceled)

55. The method of claim **23**, wherein said tumor cells are from a breast tumor, colorectal tumor, lung tumor, pancreatic tumor, prostate tumor, or a head and neck tumor.

56. The method of claim **23**, further comprising a first step to identify patients using a genetic test for treatment with an antibody that specifically binds to a non-ligand binding region of the extracellular domain of a human NOTCH receptor.

57. The method of claim **56**, wherein the genetic test detects a cancer stem cell signature.

58. A method of identifying a molecule that binds to a non-ligand binding region of an extracellular domain of a human NOTCH receptor and inhibits growth of tumor cells, the method comprising: i) incubating the molecule with the non-ligand binding domain of the extracellular domain of a human NOTCH receptor; ii) determining if the molecule binds to the non-ligand binding region of the extracellular domain of the human NOTCH receptor; and iii) determining if the molecule inhibits growth of tumor cells.

59. The method of claim **58**, wherein the non-ligand binding domain comprises EGF repeats 1-10

60. The method of claim **58** wherein the non-ligand binding domain comprises EGF repeats 13-36.

61. A pharmaceutical composition comprising the polypeptide of claim **1**.

62. An isolated nucleic acid that encodes the antibody of claim **1**.

63. An isolated nucleic acid that encodes the polypeptide of claim **21**.

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(54) **NOTCH-BASED FUSION PROTEINS AND USES THEREOF**

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(57) **ABSTRACT**

This invention provides a method for treating a subject having a tumor and a method for inhibiting angiogenesis in a subject, both comprising administering to the subject an effective amount of a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety. This invention also provides a composition of matter comprising the extracellular domain of Notch4 receptor protein operably affixed to a half-life-increasing moiety. This invention further provides an article of manufacture. Finally, this invention provides a replicable vector which encodes a polypeptide comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety, a host vector system which comprises such replicable vector and a method of producing such polypeptide.

NOTCH-BASED FUSION PROTEINS AND USES THEREOF

[0001] Vascular development begins with a process known as vasculogenesis whereby angioblasts differentiate into endothelial cells and migrate together to form the primitive vascular plexus. This initial vascular network consists of vessels that are homogenous in size and made up wholly of endothelial cells. The vascular plexus is then remodeled via angiogenesis.

[0002] Angiogenesis involves the sprouting of new vessels, the migration of these vessels into avascular regions, and the recruitment of accessory cells, pericytes and smooth muscle cells (Gale and Yancopoulos, 1999). The smooth muscle cells that differentiate and form the contractile vessel walls originate from multiple progenitors including neural crest cells, mesenchymal cells and even endothelial cells (Owens, 1995). In adults, angiogenesis is involved in follicular development, wound healing, and pathological processes such as tumor angiogenesis and heart disease.

The Notch Family and Notch Ligands

[0003] Studies of *Drosophila, C. Elegans*, zebrafish and mammals have demonstrated that the Notch pathway is an evolutionarily conserved signaling mechanism that functions to modulate numerous cell-fate decisions. Notch signaling is required for the proper patterning of cells originating from all three germ layers. Depending on the cellular context, Notch signaling may both inhibit and induce differentiation, induce proliferation, and promote cell survival (Artavanis-Tsakonas et al., 1995; Lewis, 1998; Weinmaster, 1997). In *Drosophila*, a single Notch protein is activated by two ligands, Serrate and Delta. In mammals these families have been expanded to four Notch genes (Notch1, Notch2, Notch3 and Notch4) and five ligands, 2 Serrate-like (Jagged1-2) and 3 Delta (Dll1, 3, 4) (Bettenhausen et al., 1995; Dunwoodie et al., 1997; Gallahan and Callahan, 1997; Lardelli et al., 1994; Lindsell et al., 1995; Shawber et al., 1996a; Shutter et al., 2000a; Uyttendaele et al., 1996; Weinmaster et al., 1992; Weinmaster et al., 1991). During embryogenesis, Notch receptors and ligands are expressed in dynamic spatial and temporal patterns. However, it is not known if all ligands activate all receptors.

Notch Signaling and Function

[0004] Notch signaling influences many different types of cell-fate decisions by providing inhibitory, inductive or proliferative signals depending on the environmental context (reviewed in Artavanis-Tsakonas et al., 1995; Greenwald, 1998; Robey, 1997; Vervoort et al., 1997). This pleiotropic function suggests that Notch modulates multiple signaling pathways in a spatio-temporal manner.

[0005] Consistent with Notch regulating cell-fate decisions, both the receptors and ligands are cell surface proteins with single transmembrane domains (**FIG. 1**). The regulatory extracellular domain of Notch proteins consists largely of tandemly arranged EGF-like repeats that are required for ligand binding (Artavanis-Tsakonas et al., 1995; Weinmaster, 1998). C-terminal to the EGF-like repeats are an additional three cysteine-rich repeats, designated the LIN12/Notch repeats (LNR) (Greenwald, 1994). Downstream of the LNR lies the proteolytic cleavage sequence (RXRR) that is recognized by a furin-like convertase. For Notch1, cleavage at this site yields a 180 kilodalton extracellular peptide and a 120 kilodalton intracellular peptide that are held together to generate a heterodimeric receptor at the cell surface (Blaumueller et al., 1997; Kopan et al., 1996; Logeat et al., 1998).

[0006] The intracellular domain of Notch (NotchICD, **FIG. 1**) rescues loss-of-function Notch phenotypes indicating that this form of Notch signals constitutively (Fortini and Artavanis-Tsakonas, 1993; Lyman and Young, 1993; Rebay et al., 1993; Struhl et al., 1993).

[0007] The cytoplasmic domain of Notch contains three identifiable domains: the RAM domain, the ankyrin repeat domain and the C-terminal PEST domain (**FIG. 1**). Upon ligand-activation Notch undergoes two additional proteolytic cleavages which results in the release of the cytoplasmic domain (Weinmaster, 1998). This Notch peptide translocates to the nucleus and interacts with transcriptional repressors known as CSL (CBF, Su (H), Lag-2) and converts it to transcriptional activator. The CSL/Notch interaction is dependent on the presence of the RAM domain of Notch; while, transcriptional activity also requires the presence of the ankyrin repeats (Hsieh et al., 1996; Hsieh et al., 1997; Roehl et al., 1996; Tamura et al., 1995; Wettstein et al., 1997). Both in vivo and in vitro studies indicate that the HES and Hey genes are the direct targets of Notch/CSL-dependent signaling (Bailey and Posakony, 1995; Eastman et al., 1997; Henderson et al., 2001; Jarriault et al., 1995; Nakagawa et al., 2000; Wettstein et al., 1997). The HES and Hey genes are bHLH transcriptional repressor that bind DNA at N-boxes (Nakagawa et al., 2000; Sasai et al., 1992; Tietze et al., 1992). Notch has also been proposed to signal by a CSL-independent pathway. In fact, expression of just the ankyrin repeat domain is necessary and sufficient for some forms of Notch signaling (Lieber et al., 1993; Matsuno et al., 1997; Shawber et al., 1996b).

[0008] Finally, the PEST domain has been implicated in protein turnover by a SEL-10/ubiquitin-dependent pathway (Greenwald, 1994; Oberg et al., 2001; Rogers et al., 1986; Wu et al., 1998; Wu et al., 2001). Similar to the receptors, the extracellular domain of the Notch ligands also consist mostly of tandemly arranged EGF-like repeats (**FIG. 1**). Upstream of these repeats is a divergent EGF-like repeat known as the DSL (Delta, Serrate, Lag-2) that is required for ligand binding and activation of the receptors (Artavanis-Tsakonas et al., 1995).

Notch Signaling and Vascular Development

[0009] Although many of the genes that function to induce vasculogenesis and angiogenesis have been identified, little is known about how cell-fate decisions are specified during vascular development. A number of observations suggest that the Notch signaling pathway may play a role in cell fate determination and patterning of the vascular system.

[0010] Notch1, Notch4, Jagged1 and Dll4 are all expressed in the developing vasculature, while Notch3 is expressed in the accessory smooth muscle cells (Krebs et al., 2000; Shutter et al., 2000b; Uyttendaele et al., 1996; Villa et al., 2001; Xue et al., 1999). Mice lacking Jagged1 are embryonic lethal and have severe vascular defects (Xue et al., 1999). Mice nullizygous for Notch1 are embryonic lethal and die of severe neuronal defects, but also have defects in angiogenesis (Krebs et al., 2000; Swiatek et al., 1994). Mice lacking Notch4 are born and appear to be normal, but

embryos that have lost both Notch1 and Notch4 die at E9.5 of severe hemorrhaging and vascular patterning defects indicating Notch1 and Notch4 may be functionally redundant during vascular development (Krebs et al., 2000). Exogenous expression of an activated form of Notch4 in endothelium also resulted in vascular defects similar to those seen for the double Notch1/Notch4 nullizygous mice, suggesting that appropriate levels of Notch signaling is critical for proper development of the embryonic vasculature (Uyttendaele et al., 2001).

[0011] Taken together, the data from mice mutant for Notch/Notch signaling components uncover several processes dependent on Notch including vascular remodeling, arterial venous specification, vascular smooth muscle cell recruitment and heart/heart outflow vessel development.

[0012] Recent experiments have implicated Notch signaling in arterial/venous endothelial cell specification. In situ analysis of E13.5 embryos found that Notch1, Notch3, Notch4, Dl4, Jagged1 and Jagged2 expression was restricted to the arteries and absent in the veins (Villa et al., 2001). Consistent with expression data, disruption of Notch signaling in Zebrafish was associated with loss of the arterial marker ephrinB2; while, ectopic expression of an activated form of Notch lead to a loss in the venous cell marker EphB4 within the dorsal aorta (Lawson et al., 2001). These data suggest that Notch signaling may help to specify arterial and venous cell fates during angiogenesis.

[0013] Taken together, the data from mice mutant for Notch/Notch signaling components uncover several processes dependent on Notch including vascular remodeling, arterial venous specification, vascular smooth muscle cell recruitment and heart/heart outflow vessel development.

[0014] Notch signaling has also been suggested to function in the adult vascular system. In humans, missense mutations in the extracellular domain of Notch3 correlate with the development of the degenerative vascular disease, CADASIL (Caronti et al., 1998; Desmond et al., 1998; Joutel et al., 2000; Joutel et al., 1996). In a wound healing model, an increase in Jagged1 expression was observed at the regenerating endothelial wound edge, suggesting Notch signaling may function during processes of adult angiogenesis (Lindner et al., 2001). Taken together these data support Notch signaling functions at a number of critical steps during vascular development: vasculogenesis, vascular patterning/angiogenesis, and arterial/venous specification. However, the molecular mechanism(s) by which the Notch signaling pathways influence these different steps has yet to be elucidated.

Significance

[0015] Shimizu et al. (*J. Biol. Chem.* 274(46): 32961-32969 (1999)) describe the use of Notch1ECD/Fc, Notch2ECD/Fc and Notch3ECD/Fc in binding studies. However, Shimizu et al. do not mention the use of such proteins for inhibiting angiogenesis.

[0016] U.S. Pat. No. 6,379,925 issued Apr. 30, 2002 to Kitajewsky et al. describes murine Notch4. However, it does not describe Notch-based fusion proteins as set forth in the subject application.

[0017] This invention differs from the prior art because it is the first study using Notch-based fusion proteins comprising the extracellular domain of Notch operably affixed to a half-life-increasing moiety to inhibit angiogenesis. This invention therefore provides an advantage over the prior art in that it provides evidence that such Notch-based fusion proteins are capable of inhibiting angiogenesis.

[0018] Notch proteins play key roles in developmental decisions involving the vasculature, the hematopoietic system, and the nervous system. As such, an understanding of their function is key to understanding how cell-fate decisions and commitment are controlled during development and in adult tissues. To date, several reports on Notch or Notch ligand gene disruptions have described vascular phenotypes providing emphasis that this pathway is a fundamental part of the machinery that guides vascular development. Aberrant Notch activity has been linked to human pathologies; including both cancer and vascular disorders (CADASIL). The analysis of Notch in tumor angiogenesis has only recently begun; however, our discovery of potential downstream targets of Notch suggests a roles in pathological processes associated with angiogenesis. For instance, VEGFR-3 has been linked to both tumor angiogenesis and tumor lymphangiogenesis. The expression or function of several other potential Notch targets has also been linked to tumor angiogenesis; including ephrinB2, Id3, Angiopoietin 1, and PDGF-B. Insights on the role of these targets in Notch gene function will clearly facilitate future analysis of Notch in human pathologies.

SUMMARY OF THE INVENTION

[0019] This invention provides a method for treating a subject having a tumor comprising administering to the subject an effective amount of a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety, so as to thereby treat the subject.

[0020] This invention also provides a method for inhibiting angiogenesis in a subject comprising administering to the subject an effective amount of a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety, so as to thereby inhibit angiogenesis in the subject.

[0021] This invention further provides a composition of matter comprising the extracellular domain of Notch4 receptor protein operably affixed to a half-life-increasing moiety. In one embodiment, the extracellular domain is covalently bound to the half-life-increasing moiety. In another embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

[0022] This invention further provides a composition of matter comprising the extracellular domain of Notch4 receptor protein operably affixed to a half-life-increasing moiety and a pharmaceutically acceptable carrier.

[0023] This invention further provides an article of manufacture comprising (i) a packaging material having therein a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety and (ii) a label indicating that the composition is intended for use in treating a subject having a tumor or other disorder treatable by inhibiting angiogenesis in the subject.

[0104] The terms "polypeptide,""peptide" and "protein" are used interchangeably herein, and each means a polymer of amino acid residues. The amino acid residues can be naturally occurring or chemical analogues thereof. Polypeptides, peptides and proteins can also include modifications such as glycosylation, lipid attachment, sulfation, hydroxylation, and ADP-ribosylation.

[0105] As used herein, "pharmaceutically acceptable carrier" means that the carrier is compatible with the other ingredients of the formulation and is not deleterious to the recipient thereof, and encompasses any of the standard pharmaceutically accepted carriers. Such carriers include, for example, 0.01-0.1 M and preferably 0.05 M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers can be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions and suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's and fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases, and the like.

[0106] "Subject" shall mean any organism including, without limitation, a mammal such as a mouse, a rat, a dog, a guinea pig, a ferret, a rabbit and a primate. In the preferred embodiment, the subject is a human being.

[0107] "Treating" means either slowing, stopping or reversing the progression of a disorder. As used herein, "treating" also means the amelioration of symptoms associated with the disorder.

[0108] Units, prefixes and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acid sequences are written left to right in 5' to 3' orientation and amino acid sequences are written left to right in amino- to carboxy-terminal orientation. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes.

[0109] The following abbreviations are used herein: ECD: extracellular domain; IC: intracellular domain; NECD/Fc: Notch-based fusion protein; N1: Notch1; N2: Notch2; N3: Notch3; N4: Notch4.

Embodiments of the Invention

[0110] This invention provides a first method for treating a subject having a tumor comprising administering to the subject an effective amount of a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety, so as to thereby treat the subject.

[0111] This invention also provides a second method for inhibiting angiogenesis in a subject comprising administering to the subject an effective amount of a composition of matter comprising the extracellular domain of a Notch receptor protein operably affixed to a half-life-increasing moiety, so as to thereby inhibit angiogenesis in the subject.

[0112] In a first embodiment of the above methods, the Notch receptor protein is Notch1 receptor protein. In one embodiment, the Notch1 receptor protein is human Notch1 receptor protein. In another embodiment, the half-life-increasing moiety is an Fc portion of an antibody. In another embodiment, the Fc portion of the antibody is the Fc portion of a human antibody. In a further embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

[0113] In a second embodiment of the above methods, the Notch receptor protein is Notch2 receptor protein. In one embodiment, the Notch2 receptor protein is human Notch2 receptor protein. In another embodiment, the half-life-increasing moiety is an Fc portion of an antibody. In another embodiment, the Fc portion of the antibody is the Fc portion of a human antibody. In a further embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

[0114] In a third embodiment of the above methods, the Notch receptor protein is Notch3 receptor protein. In one embodiment, the Notch3 receptor protein is human Notch3 receptor protein. In another embodiment, the half-life-increasing moiety is an Fc portion of an antibody. In another embodiment, the Fc portion of the antibody is the Fc portion of a human antibody. In a further embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

[0115] In a fourth embodiment of the above methods, the Notch receptor protein is Notch4 receptor protein. In one embodiment, the Notch4 receptor protein is human Notch4 receptor protein. In another embodiment, the half-life-increasing moiety is an Fc portion of an antibody. In another embodiment, the Fc portion of the antibody is the Fc portion of a human antibody. In a further embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

[0116] In a fifth embodiment of the above methods, the subject is a mammal. In one embodiment, the mammal is a human.

[0117] In a sixth embodiment of the above methods, the angiogenesis is tumor angiogenesis.

[0118] In a further embodiment of the second method, the subject has a tumor. In another embodiment, the subject is afflicted with a pathologic vascular hyperplasia. In one embodiment, the pathologic vascular hyperplasia is a benign hemagioma. In a further embodiment, the subject is afflicted with a lymphatic vascular proliferative disease.

[0119] This invention provides a first composition of matter comprising the extracellular domain of Notch4 receptor protein operably affixed to a half-life-increasing moiety. In one embodiment, the extracellular domain is covalently bound to the half-life-increasing moiety. In another embodiment, the extracellular domain and the half-life-increasing moiety are within the same polypeptide chain.

[0120] This invention also provides a second composition of matter comprising the extracellular domain of Notch4