

Abstract

Notch signalling is a conserved developmental pathway involved, *inter alia*, in cell-fate decision, morphogenesis and tissue patterning. Extensive research has linked this pathway with a variety of malignancies, cancer stem cell renewal, induction of epithelial-to-mesenchymal transition and tumour angiogenesis. These data indicate that Notch can act as both a tumour suppressor and an oncoprotein, depending upon cellular context and identify it as a potential therapeutic target in cancer treatment. This review discusses the implications of Notch in a number of hematologic and solid malignancies and some of the currently available inhibitors developed against this pathway as potential cancer therapeutics.

Key words: Notch, cancer, receptor, ligand, signalling

Introduction

Notch signalling is an evolutionary conserved pathway present in most multicellular organisms. It was first described by Dexter¹ who observed the appearance of a notch in the wings of *Drosophila melanogaster*. This feature became the namesake of the ‘notch’ gene and was later found to be the consequence of an X-linked, dominant mutation that causes irregular tissue loss in the wings of the fruit fly. In 1917, Morgan² identified the alleles of the gene which was later analysed and sequenced in successive years by two different groups^{3,4}.

Physiological roles of Notch signalling

The Notch pathway regulates a variety of cell fate decisions and cellular processes in invertebrate and vertebrate embryonic development and continues to remain involved in adult tissue homeostasis⁵. During embryonic development, Notch is involved in four main processes; (1) lateral inhibition which determines cell fates and spatial patterning (for example, segregation of neural and epidermal lineages or selection of a sensory organ precursor during neurogenesis)⁶, (2) asymmetric cell fate division as a mechanism to generate cell diversity and differentiate cells prior to mitosis through cell polarity regulation, (3) boundary formation – e.g. boundary establishment between prospective somites during somitogenesis or the formation of dorsal and ventral margins in the wing imaginal disc, (4) endocrine gland development through the activities of the Notch target genes *Hes1* and *Hes5*⁷⁻⁹. Notch-mediated signals are able to control divergent programs of differentiation in many tissues, including muscle, skin, pancreas and the vascular, nervous and hematopoietic systems¹⁰. In the adult body, Notch is involved in renewal and maintenance of various organs such as kidneys, lungs, liver, muscle and bone¹¹.

The Molecular biology of Notch signalling

Notch receptors – structure and maturation

Notch (Fig.1) is a 300 kDa single-pass, transmembrane protein^{4,7,12} that can act as both a receptor and a transcription factor. While *Drosophila* has only one Notch receptor, mammals express four (Notch1-4). The extracellular domains of Notch1 and Notch2 contain a tandem array of 36 epidermal growth factor (EGF)-like repeats, while Notch3 and Notch4 have 34 and 29 repeats, respectively^{4,13}. Only two of these EGF-like repeats, 11 and 12, are necessary for receptor-ligand interaction. The EGF-like repeats are followed by three cysteine-rich, LIN Notch repeats (LNRs)¹⁴. The Notch intracellular domain is also structurally complex and consists of a RBP-Jκ/CBF1 association module (RAM) domain, six ankyrin repeats, a proline, serine, glutamic acid and threonine-rich (PEST) domain and, finally, a transactivation domain (TAD) which is absent from Notch3 and Notch4¹³⁻¹⁶.

The Notch receptor is proteolytically cleaved at the S1 site (between the EGF-like and LNR repeats) whilst in the Golgi apparatus¹⁵ by a furin-like convertase (Fig.2). This event yields two fragments that are held together non-covalently through a juxtamembrane heterodimerisation domain (HD)¹⁷. Also in the Golgi apparatus, Notch is subject to glycosylation, performed by O-fucosyltransferase and Fringe^{12,13,15,18,19}. Vertebrates have three Fringe homologues; Radical, Manic and Lunatic⁸.

Canonical and non-canonical Notch signalling

The mature Notch heterodimers are held in an auto-inhibited state by a juxtamembrane negative regulatory region (NRR), located between the transmembrane and ligand-binding regions of the

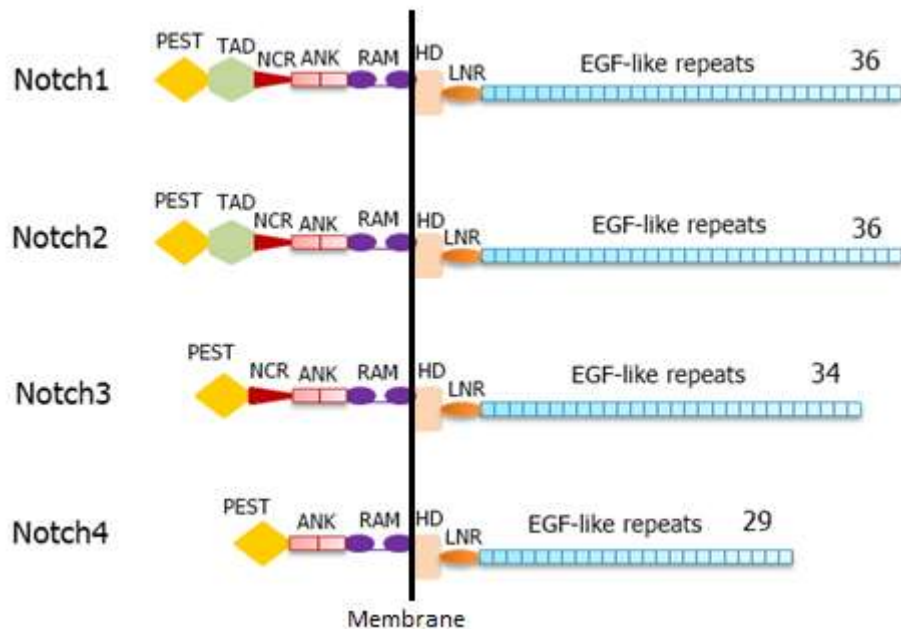


Figure 1. The modular domain structure of mammalian Notch receptors.

The extracellular domain of all Notch receptors contains multiple epidermal growth factor (EGF)-like repeats and three LIN Notch repeats (LNR) followed by the heterodimerization domain (HD). The cytosolic domain consists of a RBP-J κ /CBF1 association module (RAM) domain and several ankyrin repeats (ANK). In addition, Notch receptors 1-3 contain a Notch cytokine response (NCR) region and Notch 1 and 2 contain a transactivation domain (TAD). Finally, all four receptors possess a proline, serine, glutamic acid and threonine-rich (PEST) domain.

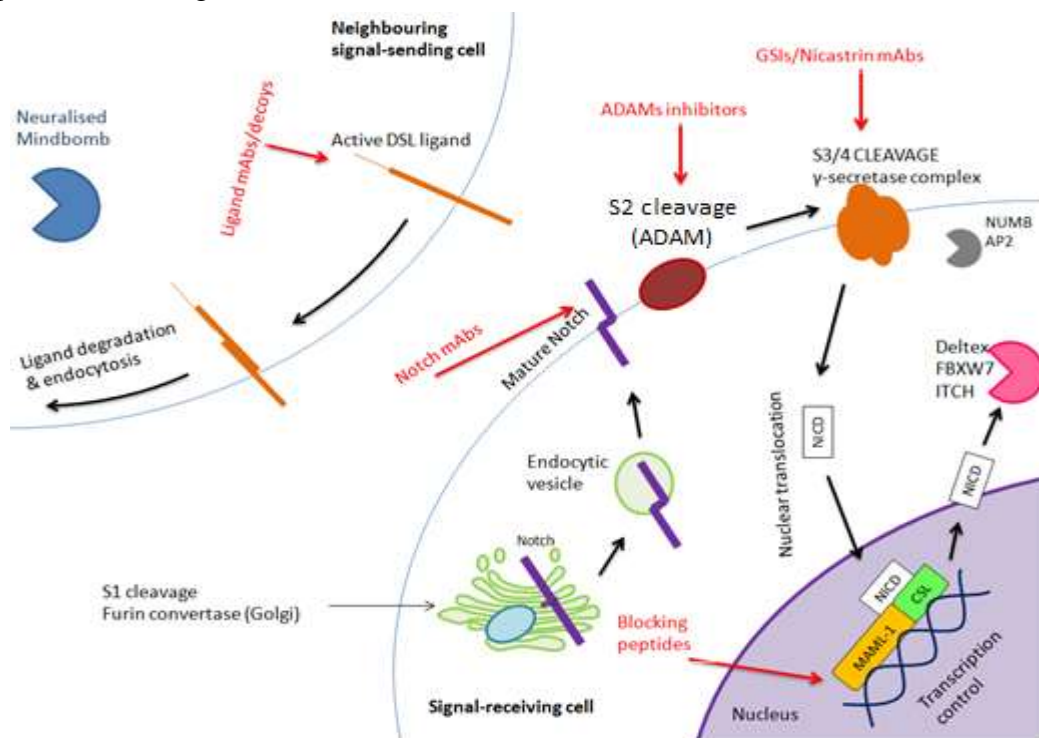


Figure 2. Trafficking and proteolysis of Notch.

The Notch receptor is proteolytically cleaved at the S1 site in the Golgi apparatus by a furin-like convertase. At the cell surface, Notch undergoes S2 cleavage by members of the ADAM family. The residual C-terminal fragment then undergoes S3/4 cleavage by the γ -secretase complex yielding the Notch intracellular domain (NICD) which translocates to the nucleus where it can modulate transcriptional events through its displacement of co-repressors associated with CSL. Mastermind-like (MAML) protein is then recruited to form a ternary complex. Possible therapeutic intervention points are shown in red.

protein^{20, 21}. This prevents activation of the Notch pathway by physically blocking the S2 cleavage site until a suitable ligand binds to the Notch receptor²⁰. Upon ligand binding, the receptor undergoes a conformational change exposing the S2 site allowing proteolytic cleavage (Fig.2)^{8, 20}. Cleavage occurs at the S2 site, between Ala-1710 and Val-1711, and is mediated by a disintegrin and metalloproteinase (ADAM) 17 also known as tumour necrosis factor alpha (TNF α)-converting enzyme (TACE)^{22, 23}.

Proteolysis of Notch by TACE is followed by cleavage at the S3 site (Fig. 2) mediated by γ -secretase, a multiprotein complex consisting of Pen2, nicastrin, Aph-1 and presenilin, with the aspartyl proteinase catalytic site located in the presenilin protein^{8, 23-25}. Nicastrin promotes the maturation and proper trafficking of other proteins in the complex^{26, 27}. S3 cleavage leads to the cytoplasmic release of Notch intracellular domain (NICD) (Fig.2) which then translocates to the nucleus²⁸.

Once in the nucleus (Fig.2), the NICD can modulate transcriptional events through its interaction with the DNA-binding factor CSL (CBF1, Suppressor of Hairless, Lag-1) that acts as both a transcriptional repressor and activator²⁹. NICD displaces the co-repressors associated with CSL and together they recruit co-activators from the Mastermind-like (MAML) protein family to form a ternary complex^{8, 30, 31}. Formation of the ternary complex depends on the ankyrin repeats of the Notch receptor^{31, 32}. Table 1 provides a list of some of the more extensively studied target genes regulated in this manner.

Table 1: Known Notch target genes and their roles (adapted^{33, 34}).

Target gene	Role/Function
Hes1, Hes5, Hes7	encode nuclear proteins that suppress transcription ³⁵
Hey1, Hey2, HeyL	encode nuclear proteins that suppress transcription ³⁶
Nanog	embryonic stem cell marker ³⁴
CD25	interleukin-2 receptor, pre-T cell receptor α chain ³³
cyclin D1	encodes a protein involved in regulating cell cycle progression ³⁷
CDK2	encodes a protein involved in regulating cell cycle progression ³⁷
DTX1	encodes Deltex-1, a E3 ubiquitin ligase ³⁸
c-Myc	proto-oncogene involved in growth control, differentiation and apoptosis ³⁹
p21 ^{WAF1}	cyclin-dependent kinase inhibitor, regulates cell cycle progression ⁴⁰
NF κ B	encodes a protein complex that controls a large number of cellular processes ⁴¹
Ifi-202, Ifi-204, Ifi-D3	encode interferon-inducible proteins ⁴²
ADAM19	a disintegrin and metalloprotease; cleaves cell surface proteins ⁴³
Bcl-2	apoptosis regulator ⁴⁴
HoxA5, HoxA9, HoxA10	regulators of animal development ⁴⁵
Slug	transcriptional repressor ⁴⁶
Survivin	apoptosis regulator ⁴⁷
NRARP	Notch negative regulator ⁴⁸
GATA3	transcription factor ⁴⁹

Protein degradation is seen as an effective means of regulating the Notch signalling pathway as it keeps the levels of the NICD just above a functional threshold⁸. The stability of the NICD is regulated by several E3 ubiquitin ligases (Deltex, Itch/AIP4, NEDD4, FBXW7 and Cbl) and polyubiquitination targets the fragment for either lysosomal degradation or recycling to the plasma membrane, both via endocytosis^{50, 51}. Numb, a cytoplasmic negative regulator of Notch that acts upstream of the γ -secretase complex, and AP2 are also able to promote Notch degradation (Fig.2)^{50, 52, 53}.

While canonical Notch signalling is able to mediate a number of biological processes, a non-canonical function of Notch has also been reported through the activity of ligands such as F3/contactin, DLK1-2, DNER and EGFL7. Non-canonical Notch is able to activate transcription independently of CSL. For instance, Notch can post-translationally target Wnt/ β -catenin signalling⁵⁴ or promote the maturation of CD4⁺ and CD8⁺ without formation of a ternary complex⁵⁰.

Notch ligands – structure and proteolysis

Canonical Notch ligands are type I transmembrane proteins, part of the DSL (Delta/Serrate/Lag2) family and only affect the activity of adjacent cells expressing the receptor⁷. The mammalian genome encodes five distinct ligands; Delta-like ligand (DLL) 1, 3 and 4 along with Jagged1 and 2^{14, 55}. Although, recently, it has been suggested that DLL3 inhibits rather than activates Notch signalling⁵⁶. The effects of certain ligands on mammalian Notch signalling may vary, depending on which of the four receptors is involved.

DSL ligands share a commonly structured extracellular region which is comprised of an N-terminal domain followed by a DSL domain and multiple EGF-like repeats (both calcium and non-calcium binding), the latter of which are essential for the interaction with Notch (Fig.3)^{5, 57}. The N-terminal domain consists of an N1 cysteine-rich region and an N2 cysteine-free region with a conserved glycosphingolipid-binding motif⁵⁸. The extracellular region of the DSL ligands differs in terms of the number of EGF-like repeats as well as the presence of a cysteine-rich region in Serrate/Jagged ligands that shares partial homology with the Von Willebrand factor. In addition, some DSL ligands present a PDZ motif⁵.

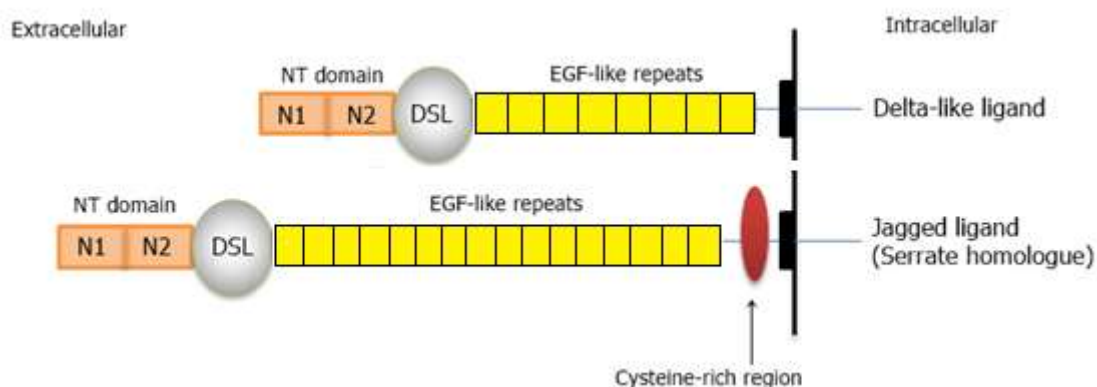


Figure 3. The modular domain structure of mammalian Notch ligands.

The mammalian genome encodes five distinct ligands; Delta-like ligand (DLL) 1, 3 and 4 along with Jagged1 and 2. Each ligand contains an epidermal growth factor (EGF)-like repeat region (15-16 repeats in Jagged ligands and 6-8 in DLLs), a DSL (Delta/Serrate/Lag2) region and N1 and N2 N-terminal regions. Additionally, the Jagged ligands also have a conserved cysteine-rich region.

Delta and Notch accumulate in endocytic vesicles and a number of recent studies have shown that ligand immobilisation and endocytosis of the extracellular domain of Delta is necessary for signal activation^{57, 59}. Like their receptors, DSL ligands undergo O- and N-linked glycan modifications¹⁹ and are proteolytically processed by ADAMs and γ -secretase⁶⁰⁻⁶³. Ligand ectodomain shedding produces membrane bound C-terminal fragments that compete with Notch for γ -secretase cleavage and lead to the loss of Notch signalling⁶². The DSL ligand intracellular domain contains multiple lysine residues that can act as sites for the attachment of ubiquitin by E3 ligases. Neutralised and Mindbomb are ubiquitin ligases that influence Notch signalling through their interaction with DSL ligands^{64, 65}. Neutralised interacts with Delta and promotes its internalisation and degradation through ubiquitination; the resultant loss of Delta at the cell surface is thought to indirectly promote Notch signalling by relieving the cis-inhibition imposed by Delta. Mindbomb also ubiquitinates and upregulates Delta endocytosis but, in contrast to Neutralised, it functions exclusively in the activation of trans-Notch signalling.

Notch signalling in cancer

Notch is one of the key pathways in embryonic development and, as such, it is not surprising that irregularities in Notch signalling have been associated with various genetic physical disorders and cancers. Examples of genetic diseases resulting from dysfunctional Notch signalling include Alagille syndrome which results from mutations in Jagged1 or Notch2^{11, 66}, spondylocostal dysostosis linked to DLL3 mutations¹⁰, and CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) caused by Notch3 mutations⁶⁷.

In terms of cancer, Notch has been shown to influence carcinogenesis through its extensive cross-talk with other signalling pathways linked to development. Notch activates the PI3kinase/Akt pathway which inhibits apoptosis⁶⁸⁻⁷⁰ and operates in an interdependent fashion with the Ras/Mitogen Activated Protein Kinase (MAPK)^{71, 72}, NF- κ B and PPAR γ pathways^{73, 74}. Furthermore, Notch also interacts with and/or influences the expression of receptor tyrosine kinases such as fibroblast growth factor receptor (FGFR)⁷⁵⁻⁷⁷, vascular endothelial growth factor receptor (VEGFR)⁷⁸⁻⁸⁰ and epidermal growth factor receptor (EGFR)^{81, 82}. Notch and the transforming growth factor- β (TGF- β) signalling pathways play critical roles during development⁸³⁻⁸⁵ and several interactions between Notch and the Wnt/ β -catenin⁸⁶⁻⁸⁸ and Hedgehog pathways have also been established^{89, 90}

Dysregulation of the Notch pathway has been associated with a wide range of solid tumours and hematologic malignancies. However, depending on the tissue and organ site in which it is expressed, the Notch pathway can be either oncogenic or tumour suppressive (Table 2).

Notch signalling in an oncogenic role

T-cell acute lymphoblastic leukaemia

T-cell acute lymphoblastic leukaemia (T-ALL) is an aggressive malignant disease affecting mainly children and adolescents. The survival rate is up to 80% but patients that relapse show a poor prognosis⁹¹. Notch signalling is essential for T-cell lineage commitment and oncogenic Notch signalling has been well documented in T-ALL^{51, 92}. In fact, more than 50% of human T-ALL cases exhibit mutations in the HD domain and/or PEST domain of Notch1 which is required during

Table 2: Oncogenic and tumour suppressive roles of Notch in a variety of cancers (adapted^{11, 13, 93}). ● Oncogene, ● Tumour suppressor, ● Oncogene and tumour suppressor

	Type of cancer	Function	Notch/Ligand	
Hematologic malignancies	Acute myeloid leukaemia	●	Notch1-2 , DLL1 and DLL4 tumour suppressor; Jagged2 oncogene	
	B-cell acute lymphoblastic leukaemia	●	Notch1-4	
	B-cell chronic lymphocytic leukaemia	●	Notch1-2, Jagged1-2	
	Chronic myelomonocytic leukaemia	●	Undetermined	
	Chronic lymphocytic leukaemia	●	5-12% Notch1 mutations	
	Diffuse large B-cell lymphoma	●	Notch2	
	Hodgkin's lymphoma	●	Notch1	
	Mantle cell lymphoma	●	~10% Notch1 mutations	
	Marginal zone lymphoma	●	Notch2	
	Multiple myeloma	●	Notch1-2, Jagged1-2 oncogenes; Notch1 tumour suppressor	
	T-cell acute lymphoblastic leukaemia	●	Notch1-3 and DLL4 oncogenes; Notch2 tumour suppressor; 60% Notch1 and 30% FBXW7 mutations	
	Solid tumours	Adenocarcinoma of the lung	●	Notch1, Notch3, Jagged2
		Breast cancer	●	Notch1-2, Notch4, Jagged1 oncogenes; Notch2 tumour suppressor
Cervical cancer		●	Notch1-2, Jagged1-2, DLL4 oncogenes; Notch1 tumour suppressor	
Cholangiocarcinoma		●	Notch1; 35% FBXW7 mutations	
Colorectal cancer		●	Notch1-2, Jagged1-2, DLL4; 8%-9% FBXW7 mutations	
Cutaneous squamous cell carcinoma		●	60-70% Notch1 and >25% Notch2 mutations	
Glioblastoma multiforme		●	Notch1-2, Jagged1, DLL1 oncogenes; Notch1 tumour suppressor	
Head and neck squamous cell carcinoma		●	15-20% Notch1 mutations; Notch1 can be an oncogene and a tumour suppressor	
Hepatocellular carcinoma		●	Notch1-2	
Lung squamous cell carcinoma		●	5-10% Notch1 mutations; Notch2	
Medulloblastoma		●	Notch1 tumour suppressor; Notch2 oncogene	
Melanoma		●	~50% Notch1 overexpression	
Non-small cell lung cancer		●	10%Notch1 mutations; Notch3; Jagged2	
Ovarian cancer		●	Undetermined	
Pancreatic cancer		●	Jagged2 (90%) and DLL4 (50%) overexpression; Notch2, Notch4 oncogene; Notch1 tumour suppressor	
Prostate cancer		●	Notch1 tumour repressor, Jagged1 oncogene	
Renal cell carcinoma		●	Undetermined	
Small cell lung cancer	●	Notch1, Notch2		

several stages of normal early T-cell development⁵¹. The majority of these mutations consist of single amino acid substitutions, insertions, and deletions. The HD domain mutations induce ligand independent signalling, while C-terminal mutations lead to the partial or complete deletion of the PEST domain and increase the half-life of the NICD^{11, 51}. Less than 1% of all T-ALL cases show a t(7;9) chromosomal translocation that results in a truncated form of the Notch1 protein through the translation of a series of truncated mRNAs. These truncated alleles lack the NRR region of the receptor and have been associated with ligand-independent activation^{16, 51, 94}.

Breast cancer

Notch plays a crucial role in mammary development and, as a result, abnormalities in the pathway lead to mammary tumourigenesis. The first indication of a link between Notch and breast cancer came with the finding that the Notch4 locus is the integration site for the mouse mammary tumour virus⁹⁵. Since then Notch has been shown to exert its oncogenic effects in breast cancer through cooperation with a number of growth promoting proteins and pathways including Ras⁷¹, enhanced cyclin A and B expression, activation of Akt signalling with an associated reduction in apoptosis, and inhibition of p53 and Foxo3a^{69, 96-98}. Notch also induces expression of the transcriptional repressor, Slug, and concomitantly promotes epithelial-to-mesenchymal transition (EMT) in E-cadherin-negative breast cancer cells⁹⁹. Furthermore, the aberrant activation of Notch signalling through the RBP-Jk pathway has been linked to human breast cancer as it leads to the accumulation of the NICD which is able to transform normal breast epithelial cells into cancerous cells¹⁰⁰. Finally, loss of Numb has been identified in more than 50% of human mammary carcinomas leading to enhanced Notch signalling¹⁰¹.

In terms of specific receptor and ligand species, the over-expression of Notch1, 3, 4 and Jagged1 has been linked to poor overall survival in breast cancer patients^{96, 98, 101-104} while Notch2 has been shown to induce apoptosis and have a tumour suppressive role in breast cancer lines¹⁰⁵. Notch3 plays an important role in the proliferation of ErbB2-negative breast tumour cells¹⁰⁶ whilst tumours expressing high levels of Notch4 appear highly vascularised and aggressive¹⁰⁵.

Melanoma

Melanomas are highly aggressive neoplasms that are unresponsive to most common therapies. The Notch pathway is important for the survival of immature melanocytes through the inhibition of apoptosis¹⁰⁷. Notch1 has been shown to enhance primary melanoma cell growth whilst having little effect on metastatic cells¹⁰⁸. The authors also showed that Notch expression was mediated by β -catenin and its functional inhibition reversed the effects of Notch on tumour growth and metastasis. The promotion of primary melanoma progression by Notch1 is also thought to occur through the activation of the MAPK/PI3-kinase/Akt pathways and the upregulation of N-cadherin expression¹⁰⁹. Dysregulated Notch1 also promotes melanomagenesis under hypoxic conditions through its interaction with PI3/Akt and NF- κ B signalling. Low oxygen is able to upregulate Notch1 signalling via stabilisation of HIF-1 α (hypoxia-inducible factor-1 α)⁶⁸.

Notch signalling in a tumour suppressor role

Skin carcinomas

In contrast to its oncogenic role in melanoma progression, Notch1 has been identified as a tumour suppressor in mouse basal cell and squamous cell carcinomas of the skin where it interacts with the Wnt and Hedgehog pathways^{108, 110}. In keratinocytes, Notch signalling is able to promote

differentiation and suppress tumourigenesis. Activated Notch1 leads to keratinocyte growth arrest by increasing p21^{WAF1/Cip1} expression (cyclin/CDK inhibitor) through RBP-jK-dependent transcription¹¹¹. p21 also acts as a negative transcriptional regulator of Wnt expression downstream of Notch1¹¹². Notch1 can also suppress the expression of p63 (a modulator of Notch1-dependent transcription) in both human and murine keratinocytes¹¹³. Deletion of Notch1 results in epidermal and corneal hyperplasia¹¹¹ while Notch1 deficiency is associated with the upregulation of Gli2 which leads to the development of basal-cell tumours¹¹⁴.

Small cell lung carcinoma

Small cell lung carcinoma (SCLC) is a neuroendocrine subtype of lung cancer with a high mortality rate. Notch signalling has a tumour suppressive role in SCLC as indicated by the loss-of-function mutations in human tumours. The Notch family of receptors is affected by genomic alterations in 25% of SCLC cases¹¹⁵. The majority of the identified mutations are heterozygous and mainly cluster in the EGF-like repeat region of Notch1. Most aberrations are frameshift and nonsense mutations as well as substitutions¹¹⁶. Notch signalling is able to cause growth arrest associated with G₁ cell cycle block and upregulate the expression of p21^{waf/cip1} and p27^{kip1} in SCLC cancer cell lines with cycle arrest being linked to the repression of HASH1 and induction of the MAPK/Ras pathway¹¹⁷.

Notch signalling and tumour angiogenesis

Tumour growth and metastasis is dependent on angiogenesis which is controlled by multiple signalling mechanisms such as the VEGF, FGF and hepatocyte growth factor (HGF) pathways. The Notch signalling pathway plays a pivotal role in vascular development and tumour angiogenesis, from vessel maturation, branching and cell differentiation to cell proliferation, survival and apoptosis^{78, 118}. The VEGF and Notch pathways have independent but complementary functions in tumour angiogenesis; VEGF can stimulate the expression of Notch receptors and ligands while Notch is able to regulate the expression of VEGFs^{79, 118, 119}. DLL4 haploinsufficiency leads to severe vascular defects in embryos and increased expression of the ligand is associated with human cancers^{120, 121}. Many studies have reported that DLL4 acts as a negative regulator of tumour angiogenesis by disrupting vessel density and structure¹²²⁻¹²⁴. Some data, however, suggest that inhibition of the DLL4/Notch pathway induces vascular neoplasms^{79, 119, 125}.

Notch signalling and cancer stem cells

Stem cells are characterised by their capacity for self-renewal and increasing data point toward the existence of cancer stem cells (CSCs). These 'tumour-initiating cells' are self-sustaining and capable of indefinite self-replication all the while showing resistance to chemotherapy and radiation which means that their complete eradication is necessary to obtain a cure for cancer. The Notch pathway, along with Wnt and Hedgehog, is essential for the maintenance of this population of cells and contributes to inflammatory signalling which promotes the stem-like cell phenotype^{98, 126}. Recent work has shown that Notch plays a central role in breast and glioma CSCs. Notch1-transformed mouse mammary tumors have been shown to harbour a rare mammary tumor-initiating cell population and the receptor contributes to mammary tumour-initiating activity¹²⁷. The authors demonstrated that Notch1 over-expression was able to increase the rate of tumoursphere formation in murine mammary tumour cell cultures through expression of the embryonic stem cell transcription factor, Nanog. Notch4 signalling has also been shown to be enhanced 8-fold in breast CSCs¹⁰³. In glioblastoma models, knockdown of Notch ligands through RNA interference was found to hinder CSC self-renewal and growth¹²⁸.

Strategies to regulate Notch signalling for cancer therapy

The range of roles exhibited by Notch signalling in tumorigenesis, angiogenesis and CSC maintenance makes it a viable therapeutic target for cancer. Inhibition of the Notch pathway with various agents from small molecule inhibitors to large molecule antibodies is being actively investigated as there is a need to identify new, less toxic and more efficacious disease treatments.

Notch-related antibodies

Molecules that target Notch receptors or ligands in a specific manner should, theoretically, reduce the therapeutic complications that arise from using non-selective compounds in cancer treatment. An attractive prospect in this respect is that of monoclonal antibody (mAb) therapy. However, monoclonal antibodies cannot cross the blood-brain barrier so they cannot, unmodified, be used against primary brain tumours and metastases and have a short half-life.

Using phage display technology, mAbs that can recognise specific ligands and receptors have been generated⁵⁰. These antibodies can act as potent inhibitors of the Notch pathway by preventing ligand-receptor interaction or proteolytic cleavage, thus inhibiting the production of the NICD^{96, 129}. Aste-Amezaga *et al.*¹²⁹ generated two types of mAb against Notch1; one of which recognized the NRR of the receptor and the other of which interacted with the ligand binding domain. Both antibodies were able to downregulate Notch signalling with the NRR-specific antibody having anti-angiogenic effects on tumours. However, the same authors noted that dual Notch1 and Notch2 mAb-mediated inhibition caused gastrointestinal toxicity. Antibodies that bind to overlapping epitopes in the NRR region of Notch3 have been shown to inhibit the pathway by stabilising the auto-inhibited state and preventing proteolysis²¹ whilst the antibody, OMP-59R5 (tarextumab), which is a Notch2/Notch3 antagonist, has shown promising results in various cancer models, both as a single agent or in combination with other drugs¹³⁰.

Antibodies against Notch ligands are also under development. An anti-DLL4 antibody¹²⁴ and a soluble Dll4-Fc fusion protein^{123, 131} have been shown to have anti-tumour activity that disrupts angiogenesis and inhibits tumour growth. OMP-21M18 is an antibody against DLL4 that blocks ligand interaction with Notch1 and Notch4 thus inhibiting the signalling pathway. Multiple early-stage clinical trials are being conducted to test the efficacy of OMP-21M18 as a single agent or in combination with chemotherapy^{13, 132, 133}

Finally, A5226A, an antibody against the extracellular domain of nicastrin (a component of the γ -secretase complex), is able to neutralise the activity of γ -secretase without causing any off-target effects¹³⁴.

Notch signalling decoys

Soluble decoys of the extracellular domain of Notch receptors and ligands appear to inhibit signalling. Funahashi *et al.*¹²¹ employed a construct containing the 36 EGF repeats of rat Notch1 fused to human Fc and demonstrated that it blocked Notch signalling in endothelial cells and impaired tumour neoangiogenesis with a 58% decrease in microvessel density in xenograft models. Monomeric and dimeric forms of DLL-1 generated by fusing the extracellular domain to either a series of myc epitopes or to the Fc portion of human IgG-1 have also been shown to impair the activation of Notch by tethered DLL-1, suggesting a direct competition between soluble and tethered ligands⁵⁹. Similarly, soluble Jagged1 has been shown to repress the function of its transmembrane counterpart¹³⁵. Although not strictly a decoy, the soluble protein, epidermal growth factor-like domain 7 (EGFL7), has been shown to suppress endothelial cell proliferation, sprouting and migration in a manner reminiscent of Notch inhibition¹³⁶. Furthermore, the authors

demonstrated a physical interaction between Notch and EGFL7 suggesting that the latter protein might compete with Notch-ligand binding to inhibit angiogenesis.

γ -secretase inhibitors

Proteolytic cleavage of Notch receptors and/or ligands by the γ -secretase complex is a prerequisite for the downstream transcriptional changes associated with Notch signalling. γ -secretase inhibitors (GSIs), therefore, inhibit Notch signalling by reducing the formation of the NICD. These compounds have cytostatic and cytotoxic activities in various cancer cells. However, the drawback of these effects is that most GSIs are not highly specific as they impair the proteolysis of a range of γ -secretase substrates including DLLs, Jagged, CD44, E-cadherin, amyloid precursor protein and N-cadherin. Two classes of GSIs have been developed with differing specificities; non-transition state inhibitors and competitive inhibitors of the presenilin catalytic site¹³³.

RO4929097 is a competitive oral GSI that shows anti-tumour activity in multiple xenograft models. The inhibitor does not block proliferation or induce apoptosis but instead it produces a less transformed phenotype^{137, 138}. The compound can impair angiogenesis but its effects in this respect are limited by high tumour levels of IL6 and IL8¹³⁹. RO4929097 has been shown to downregulate the expression of Notch target genes in breast cancer cell lines¹²⁶ and to reduce the tumour initiating potential of melanoma cells^{140, 141}. In addition, it has also been shown to reduce the expression of Notch target genes (Hes1, 3 and 5) in a dose-dependent manner in glioma tumour-initiating cells¹⁴². However, it was later shown that RO4929097 had limited anti-tumour activity in established glial tumours but that its efficacy was enhanced when used in combination with various other established chemotherapeutic agents¹⁴³. Similarly, a phase 1 study of patients with a range of refractory solid tumours (predominantly colon) demonstrated a clinical benefit in colon and cervical cancer patients when RO4929097 was used in combination with capecitabine¹⁴⁴. The former compound seemed to enhance the sensitivity of tumour cells to capecitabine. However, common side effects included grade 3 and 4 toxicities in relation to nausea, vomiting, diarrhoea, fatigue and hypophosphatemia.

PF-03084014 is a non-competitive and selective GSI that has been shown to reduce endogenous NICD levels and downregulate the Notch target genes Hes-1 and cMyc in the T-ALL cell line HPB-ALL¹⁴⁵. The authors demonstrated growth inhibition of several T-ALL cell lines via cell cycle arrest and the induction of apoptosis. Furthermore, the inhibitor reduced cell proliferation and induced apoptosis in HPB-ALL tumours. Used in combination with fludarabine, PF-03084014 induced selective apoptosis in Notch1-mutated chronic lymphocytic leukaemia cells and upregulation of HRK, a proapoptotic gene¹⁴⁶. Furthermore, the inhibitor has been shown to exhibit synergistic activity with dexamethasone (a glucocorticoid) in human T-ALL cell lines and primary human T-ALL patient samples¹⁴⁷. The authors also demonstrated that the combination of compounds was highly efficacious in reducing the tumour burden in a xenograft model of T-ALL and that dexamethasone ablated the gastrointestinal toxicity of PF-03084014. Similarly, synergistic activity of PF-03084014 with docetaxel has been shown in triple-negative breast cancer models. The combination of the two drugs reversed the endothelial mesenchymal transition phenotype, induced apoptosis in bulk tumours and eliminated cancer stem cells¹⁴⁸.

DAPT (N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester / GSI-IX) is a GSI that has been widely used to evaluate the role of Notch signalling in various cell types such as muscle stem cells, neural stem cells, ovarian cancer cells and tongue carcinoma cells^{80, 149, 150}. DAPT blocks cellular proliferation and induces apoptosis through expression of p21 and regulation of cyclin A in Ishikawa endometrial cancer cells¹⁵¹ and reduces proliferation in adipose derived stem cells via regulation of Notch and Runx2 expression¹⁵⁰. DAPT can also inhibit Notch1 in gastric cancer cells which in turn leads to EMT inhibition¹⁵². Synergistic anti-leukaemic activity was observed following the pretreatment of ovarian cell lines resistant to cisplatin with DAPT¹⁵³. Furthermore, the authors demonstrated that the drug combination inhibited tumour growth and

induced G2 cell cycle arrest and apoptosis through modulation of cyclin B1, Bcl-2 and caspase-3. DAPT showed no effect on tumour angiogenesis in colon adenocarcinoma in obese mice¹⁵⁴ but it was able to interfere with VEGF signalling in glioblastomas by decreasing VEGFR1 expression which led to uncoupling of tumour vessel density from vessel function⁸⁰. However, the authors also showed that DAPT increased VEGFR2 expression and enhanced endothelial cell proliferation in combination with VEGF treatment.

MRK-003 is a potent GSI that exhibits good preclinical activity in several T-ALL and breast cancer cell lines. Intermittent treatment (3 days per week) is sufficient to induce Notch1-mediated cell cycle arrest and apoptosis in T-ALL¹⁵⁵. Combining MRK-003 with trastuzumab (Herceptin) prevents ErbB-2 positive breast tumour recurrence while a combination of MRK-003 with lapatinib significantly reduces tumour growth¹⁵⁶. MRK-003 treatment promotes caspase-dependent apoptosis and blocks proliferation in non-Hodgkin's lymphoma and multiple myeloma cell lines by decreasing the levels of NICD, Hes1 and cMyc¹⁵⁷. Moreover, the authors demonstrated that the inhibitor upregulated pAkt expression while downregulating the levels of p21, Bcl-2 and Bcl-XI in multiple myeloma cells and cyclin D1, Xiap and Bcl-XI in non-Hodgkin's lymphoma cells. MRK-003 has also been shown to cause cytotoxicity and growth inhibition in a mouse model of pancreatic ductal adenocarcinoma¹⁵⁸ but appears to reduce oxaliplatin-induced apoptosis in human colon cancer cells by increasing the levels of the anti-apoptotic proteins Mc1-1 and Bcl-xL¹⁵⁹.

Compound E is a selective, non-competitive GSI that has shown anti-tumour activity in several pre-clinical studies. The treatment of T-ALL cell lines with Compound E for 5-7 days has been shown to reversibly inhibit cell proliferation, cause cell cycle block and differentiation (the latter only in some of the cell lines studied)¹⁶⁰. Treatment of 14 days or longer was required to induce significant apoptosis but the authors also found that Compound E sensitized cells to the effects of dexamethasone raising the potential for the use of the drug combination to obtain efficient therapeutic effects in T-ALL. Another study tested the response of compound E in four T-ALL cell lines in combination with several chemotherapeutic drugs. GSI treatment showed inconsistencies: it led to downregulation of Notch in one line, had no effect on another and induced chemotherapy resistance in two of the cell lines tested⁹¹.

GSI-I can induce cell cycle arrest and apoptosis in breast cancer cells with estrogen receptor (ER)-negative cell lines showing increased sensitivity²⁶. Treatment of precursor-B acute lymphoblastic leukaemia (ALL) blasts with GSI-I induced apoptosis and caused nuclear accumulation of cleaved Notch1 and Notch2 concomitant with an inhibition of the Notch targets Hey2 and Myc¹⁶¹

MK-0752 is a novel GSI that has shown promising early results. The drug has been shown to reduce breast cancer stem cell numbers in tumourgrafts and to enhance the efficacy of docetaxel in pre-clinical studies¹⁶². In a 20-patient pilot study, MK-0752 was tested against early stage ER+ breast cancer and showed significant biomarker response in all tumours through modulation of Notch activity¹⁶³. In another study investigating the effect of MK-0752 on advanced solid tumours, the inhibitor significantly impaired Notch signalling and a limited proportion of patients showed disease stabilization for longer than 4 months (one showed a complete response)¹⁶⁴.

Although GSIs show promising results in clinical studies, they fail to distinguish between individual Notch receptors and they inhibit other signalling pathways that utilise the γ -secretase complex. This lack of specificity can lead to gastrointestinal toxicity due to the rapid differentiation of progenitor cells into secretory goblet cells in the intestinal crypts (goblet cell metaplasia) being impaired by Notch inhibition. Other GSI-associated adverse effects include skin disorders such as erythema, rash and pruritus or headaches^{96, 133, 165}. However, as mentioned earlier, some studies have demonstrated that the combination of GSIs with other drugs such as glucocorticoids might overcome some of these toxicity problems¹⁴⁷.

Blocking peptides

Agents that interfere with the Notch signalling transcriptional activator complex have also been generated. SAHM1 is a permeable peptide that directly antagonises the assembly of the ternary complex and causes potent suppression of Notch target genes. SAHM1 blocks MAML1 recruitment and forms a transcriptionally inert complex with Notch and CSL. This inhibits Notch signalling and blocks tumour growth without showing gastrointestinal side effects¹⁶⁶. TR4 is another dominant negative peptide derived from MAML1 that has shown promising results in human mammary and colon xenograft models. Moreover, the compound is able to cross the blood-brain barrier so it can potentially be used against brain tumours¹⁶⁷.

Concluding remarks

A plethora of studies have demonstrated how aberrant Notch signalling increases cellular proliferation, induces epithelial-to-mesenchymal transition, maintains the cancer stem cell pool and inhibits apoptosis. The multifaceted role of Notch in cancer development/progression makes it an attractive therapeutic target and its cross-talk with other signalling pathways opens the door for the use of combinational therapeutic strategies. However, the efficacy of any Notch-targeted treatments must be considered in the light of drug specificity and related off-target toxicities. Furthermore, the role Notch signalling in cancer, as in differentiation, is context dependent; it can promote oncogenesis in some tissues and prevent neoplastic transformation in others. Nonetheless, the use of drugs to regulate Notch signalling represents an exciting challenge in the field of cancer therapeutics.

Abbreviations

ADAM, a disintegrin and metalloproteinase; CADASIL, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CSC, cancer stem cell; CSL, CBF1 Suppressor of Hairless, Lag-1; DAPT, N-[N-(3,5-Difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; DLL, Delta-like ligand; DSL, Delta/Serrate/Lag2; EGF, epidermal growth factor; EGFL7, epidermal factor-like domain 7; EGFR, epidermal growth factor receptor; EMT, epithelial-to-mesenchymal transition; ER, estrogen receptor; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GSI, γ -secretase inhibitor; HD, heterodimerisation domain; HGF, hepatocyte growth factor; HIF-1 α , hypoxia-inducible factor-1 α ; LNR, LIN-12-Notch repeat; mAb, monoclonal antibody; MAML, Mastermind-like; MAPK, Ras/Mitogen Activated Protein Kinase; NICD, Notch intracellular domain; NRR, negative regulatory region; PEST, proline, serine, glutamic acid and threonine-rich; RAM, RBP-J κ /CBF1 association module; SCLC, small cell lung carcinoma; T-ALL, T-cell acute lymphoblastic leukaemia; TAD, transactivation domain; TACE, tumour necrosis factor alpha-converting enzyme; TGF- β , transforming growth factor- β ; TNF α , tumour necrosis factor alpha; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor

References

1. Dexter J. The analysis of a case of continuous variation in *Drosophila* by a study of its linkage relations. *The American Naturalist*. 1914. 48(576): 712.
2. Morgan TH. The theory of the gene. *The American Naturalist*. 1917. 51(609): 513-544.
3. Kidd S, Kelley MR, Young MW. Sequence of the notch locus of *Drosophila melanogaster*: relationship of the encoded protein to mammalian clotting and growth factors. *Mol Cell Biol*. 1986. 6(9): 3094-108.
4. Wharton KA, Johansen KM, Xu T, Artavanis-Tsakonas S. Nucleotide sequence from the neurogenic locus notch implies a gene product that shares homology with proteins containing EGF-like repeats. *Cell*. 1985. 43(3 Pt 2): 567-81.
5. D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. *Curr Top Dev Biol*. 2010. 92: 73-129.
6. Luo B, Aster JC, Hasserjian RP, Kuo F, Sklar J. Isolation and functional analysis of a cDNA for human Jagged2, a gene encoding a ligand for the Notch1 receptor. *Mol Cell Biol*. 1997. 17(10): 6057-67.
7. Artavanis-Tsakonas S, Rand M, Lake R. Notch signaling: cell fate control and signal integration in development. *Science*. 1999. 284(5415): 770-6.
8. Fiuza UM, Arias AM. Cell and molecular biology of Notch. *J Endocrinol*. 2007. 194(3): 459-74.
9. Muskavitch MA. Delta-notch signaling and *Drosophila* cell fate choice. *Dev Biol*. 1994. 166(2): 415-30.
10. Hansson EM, Lendahl U, Chapman G. Notch signaling in development and disease. *Semin Cancer Biol*. 2004. 14(5): 320-8.
11. Ntziachristos P, Lim JS, Sage J, Aifantis I. From fly wings to targeted cancer therapies: a centennial for notch signaling. *Cancer Cell*. 2014. 25(3): 318-34.
12. Logeat F, Bessia C, Brou C *et al*. The Notch1 receptor is cleaved constitutively by a furin-like convertase. *Proc Natl Acad Sci U S A*. 1998. 95(14): 8108-12.
13. Previs RA, Coleman RL, Harris AL, Sood AK. Molecular pathways: translational and therapeutic implications of the Notch signaling pathway in cancer. *Clin Cancer Res*. 2015. 21(5): 955-61.
14. Rebay I, Fleming RJ, Fehon RG, Cherbas L, Cherbas P, Artavanis-Tsakonas S. Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor. *Cell*. 1991. 67(4): 687-99.
15. Blaumueller CM, Qi H, Zagouras P, Artavanis-Tsakonas S. Intracellular cleavage of Notch leads to a heterodimeric receptor on the plasma membrane. *Cell*. 1997. 90(2): 281-91.
16. South AP, Cho RJ, Aster JC. The double-edged sword of Notch signaling in cancer. *Semin Cell Dev Biol*. 2012.
17. Malecki MJ, Sanchez-Irizarry C, Mitchell JL *et al*. Leukemia-associated mutations within the NOTCH1 heterodimerization domain fall into at least two distinct mechanistic classes. *Mol Cell Biol*. 2006. 26(12): 4642-51.
18. Okajima T, Irvine KD. Regulation of notch signaling by o-linked fucose. *Cell*. 2002. 111(6): 893-904.
19. Shi S, Stanley P. Protein O-fucosyltransferase 1 is an essential component of Notch signaling pathways. *Proc Natl Acad Sci U S A*. 2003. 100(9): 5234-9.
20. Gordon WR, Vardar-Ulu D, Histen G, Sanchez-Irizarry C, Aster JC, Blacklow SC. Structural basis for autoinhibition of Notch. *Nat Struct Mol Biol*. 2007. 14(4): 295-300.
21. Li K, Li Y, Wu W *et al*. Modulation of Notch signaling by antibodies specific for the extracellular negative regulatory region of NOTCH3. *J Biol Chem*. 2008. 283(12): 8046-54.
22. Brou C, Logeat F, Gupta N *et al*. A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell*. 2000. 5(2): 207-16.

23. Mumm JS, Schroeter EH, Saxena MT *et al.* A ligand-induced extracellular cleavage regulates gamma-secretase-like proteolytic activation of Notch1. *Mol Cell*. 2000. 5(2): 197-206.
24. De Strooper B, Annaert W, Cupers P *et al.* A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. *Nature*. 1999. 398(6727): 518-22.
25. Six E, Ndiaye D, Laabi Y *et al.* The Notch ligand Delta1 is sequentially cleaved by an ADAM protease and gamma-secretase. *Proc Natl Acad Sci U S A*. 2003. 100(13): 7638-43.
26. Al-Hussaini H, Subramanyam D, Reedijk M, Sridhar SS. Notch signaling pathway as a therapeutic target in breast cancer. *Mol Cancer Ther*. 2011. 10(1): 9-15.
27. Shah S, Lee SF, Tabuchi K *et al.* Nicastrin functions as a gamma-secretase-substrate receptor. *Cell*. 2005. 122(3): 435-47.
28. Schroeter EH, Kisslinger JA, Kopan R. Notch-1 signalling requires ligand-induced proteolytic release of intracellular domain. *Nature*. 1998. 393(6683): 382-6.
29. Kao HY, Ordentlich P, Koyano-Nakagawa N *et al.* A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. *Genes Dev*. 1998. 12(15): 2269-77.
30. Nam Y, Sliz P, Song L, Aster JC, Blacklow SC. Structural basis for cooperativity in recruitment of MAML coactivators to Notch transcription complexes. *Cell*. 2006. 124(5): 973-83.
31. Petcherski AG, Kimble J. Mastermind is a putative activator for Notch. *Curr Biol*. 2000. 10(13): R471-3.
32. Weng AP, Nam Y, Wolfe MS *et al.* Growth suppression of pre-T acute lymphoblastic leukemia cells by inhibition of notch signaling. *Mol Cell Biol*. 2003. 23(2): 655-64.
33. Borggreffe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci*. 2009. 66(10): 1631-46.
34. Capaccione KM, Pine SR. The Notch signaling pathway as a mediator of tumor survival. *Carcinogenesis*. 2013. 34(7): 1420-30.
35. Kageyama R, Ohtsuka T, Kobayashi T. The Hes gene family: repressors and oscillators that orchestrate embryogenesis. *Development*. 2007. 134(7): 1243-51.
36. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol*. 2003. 194(3): 237-55.
37. Ronchini C, Capobianco AJ. Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol*. 2001. 21(17): 5925-34.
38. Zhang P, Yang Y, Nolo R, Zweidler-McKay PA, Hughes DP. Regulation of NOTCH signaling by reciprocal inhibition of HES1 and Deltex 1 and its role in osteosarcoma invasiveness. *Oncogene*. 2010. 29(20): 2916-26.
39. Hoffman B, Liebermann DA. Apoptotic signaling by c-MYC. *Oncogene*. 2008. 27(50): 6462-72.
40. Cmielova J, Rezacova M. p21Cip1/Waf1 protein and its function based on a subcellular localization [corrected]. *J Cell Biochem*. 2011. 112(12): 3502-6.
41. Gilmore TD. Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene*. 2006. 25(51): 6680-4.
42. Landolfo S, Gariglio M, Gribaudo G, Lembo D. The Ifi 200 genes: an emerging family of IFN-inducible genes. *Biochimie*. 1998. 80(8-9): 721-8.
43. Qi B, Newcomer RG, Sang QX. ADAM19/adamalysin 19 structure, function, and role as a putative target in tumors and inflammatory diseases. *Curr Pharm Des*. 2009. 15(20): 2336-48.
44. Cory S, Huang DC, Adams JM. The Bcl-2 family: roles in cell survival and oncogenesis. *Oncogene*. 2003. 22(53): 8590-607.
45. P.Z. M. Hox genes in development; the Hox code. *Nature Education*. 2008. 1(1): 2.

46. Medici D, Hay ED, Olsen BR. Snail and Slug promote epithelial-mesenchymal transition through beta-catenin-T-cell factor-4-dependent expression of transforming growth factor-beta3. *Mol Biol Cell*. 2008. 19(11): 4875-87.
47. Fukuda S, Pelus LM. Survivin, a cancer target with an emerging role in normal adult tissues. *Mol Cancer Ther*. 2006. 5(5): 1087-98.
48. Lamar E, Deblandre G, Wettstein D *et al*. Nrarp is a novel intracellular component of the Notch signaling pathway. *Genes Dev*. 2001. 15(15): 1885-99.
49. Wang Y, Su MA, Wan YY. An essential role of the transcription factor GATA-3 for the function of regulatory T cells. *Immunity*. 2011. 35(3): 337-48.
50. Espinoza I, Miele L. Notch inhibitors for cancer treatment. *Pharmacol Ther*. 2013. 139(2): 95-110.
51. Weng AP, Aster JC. Multiple niches for Notch in cancer: context is everything. *Curr Opin Genet Dev*. 2004. 14(1): 48-54.
52. Bray SJ. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol*. 2006. 7(9): 678-89.
53. Le Borgne R. Regulation of Notch signalling by endocytosis and endosomal sorting. *Current Opinion in Cell Biology*. 2006. 18(2): 213-222.
54. Andersen P, Uosaki H, Shenje LT, Kwon C. Non-canonical Notch signaling: emerging role and mechanism. *Trends Cell Biol*. 2012. 22(5): 257-65.
55. Lindsell CE, Shawber CJ, Boulter J, Weinmaster G. Jagged: a mammalian ligand that activates Notch1. *Cell*. 1995. 80(6): 909-17.
56. Ladi E, Nichols JT, Ge W *et al*. The divergent DSL ligand Dll3 does not activate Notch signaling but cell autonomously attenuates signaling induced by other DSL ligands. *J Cell Biol*. 2005. 170(6): 983-92.
57. Chitnis A. Why is delta endocytosis required for effective activation of notch? *Dev Dyn*. 2006. 235(4): 886-94.
58. Hamel S, Fantini J, Schweisguth F. Notch ligand activity is modulated by glycosphingolipid membrane composition in *Drosophila melanogaster*. *J Cell Biol*. 2010. 188(4): 581-94.
59. Varnum-Finney B, Wu L, Yu M *et al*. Immobilization of Notch ligand, Delta-1, is required for induction of notch signaling. *J Cell Sci*. 2000. 113 Pt 23: 4313-8.
60. Bland CE, Kimberly P, Rand MD. Notch-induced proteolysis and nuclear localization of the Delta ligand. *J Biol Chem*. 2003. 278(16): 13607-10.
61. Dyczynska E, Sun D, Yi H, Sehara-Fujisawa A, Blobel CP, Zolkiewska A. Proteolytic processing of delta-like 1 by ADAM proteases. *J Biol Chem*. 2007. 282(1): 436-44.
62. LaVoie MJ, Selkoe DJ. The Notch ligands, Jagged and Delta, are sequentially processed by alpha-secretase and presenilin/gamma-secretase and release signaling fragments. *J Biol Chem*. 2003. 278(36): 34427-37.
63. Parr-Sturgess CA, Rushton DJ, Parkin ET. Ectodomain shedding of the Notch ligand Jagged1 is mediated by ADAM17, but is not a lipid-raft-associated event. *Biochem J*. 2010. 432(2): 283-94.
64. Koo BK, Yoon MJ, Yoon KJ *et al*. An obligatory role of mind bomb-1 in notch signaling of mammalian development. *PLoS One*. 2007. 2(11): e1221.
65. Song R, Koo BK, Yoon KJ *et al*. Neuralized-2 regulates a Notch ligand in cooperation with Mind bomb-1. *J Biol Chem*. 2006. 281(47): 36391-400.
66. Li L, Krantz ID, Deng Y *et al*. Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. *Nat Genet*. 1997. 16(3): 243-51.
67. Joutel A, Corpechot C, Ducros A *et al*. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature*. 1996. 383(6602): 707-10.
68. Bedogni B, Warneke JA, Nickoloff BJ, Giaccia AJ, Powell MB. Notch1 is an effector of Akt and hypoxia in melanoma development. *J Clin Invest*. 2008. 118(11): 3660-70.

69. Meurette O, Stylianou S, Rock R, Collu GM, Gilmore AP, Brennan K. Notch activation induces Akt signaling via an autocrine loop to prevent apoptosis in breast epithelial cells. *Cancer Res.* 2009. 69(12): 5015-22.
70. Rangarajan A, Syal R, Selvarajah S, Chakrabarti O, Sarin A, Krishna S. Activated Notch1 signaling cooperates with papillomavirus oncogenes in transformation and generates resistance to apoptosis on matrix withdrawal through PKB/Akt. *Virology.* 2001. 286(1): 23-30.
71. Weijzen S, Rizzo P, Braid M *et al.* Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. *Nat Med.* 2002. 8(9): 979-86.
72. Zeng Q, Li S, Chepeha DB *et al.* Crosstalk between tumor and endothelial cells promotes tumor angiogenesis by MAPK activation of Notch signaling. *Cancer Cell.* 2005. 8(1): 13-23.
73. Joshi I, Minter LM, Telfer J *et al.* Notch signaling mediates G1/S cell-cycle progression in T cells via cyclin D3 and its dependent kinases. *Blood.* 2009. 113(8): 1689-98.
74. Nickoloff BJ, Qin JZ, Chaturvedi V, Denning MF, Bonish B, Miele L. Jagged-1 mediated activation of notch signaling induces complete maturation of human keratinocytes through NF-kappaB and PPARgamma. *Cell Death Differ.* 2002. 9(8): 842-55.
75. Di Giovanni V, Walker KA, Bushnell D *et al.* Fibroblast growth factor receptor-Frs2alpha signaling is critical for nephron progenitors. *Dev Biol.* 2015. 400(1): 82-93.
76. Small D, Kovalenko D, Soldi R *et al.* Notch activation suppresses fibroblast growth factor-dependent cellular transformation. *J Biol Chem.* 2003. 278(18): 16405-13.
77. Yoon K, Nery S, Rutlin ML, Radtke F, Fishell G, Gaiano N. Fibroblast growth factor receptor signaling promotes radial glial identity and interacts with Notch1 signaling in telencephalic progenitors. *J Neurosci.* 2004. 24(43): 9497-506.
78. Liu ZJ, Shirakawa T, Li Y *et al.* Regulation of Notch1 and Dll4 by vascular endothelial growth factor in arterial endothelial cells: implications for modulating arteriogenesis and angiogenesis. *Mol Cell Biol.* 2003. 23(1): 14-25.
79. Shawber CJ, Funahashi Y, Francisco E *et al.* Notch alters VEGF responsiveness in human and murine endothelial cells by direct regulation of VEGFR-3 expression. *J Clin Invest.* 2007. 117(11): 3369-82.
80. Zou Y, Cao Y, Yue Z, Liu J. Gamma-secretase inhibitor DAPT suppresses glioblastoma growth via uncoupling of tumor vessel density from vessel function. *Clin Exp Med.* 2013. 13(4): 271-8.
81. Aguirre A, Rubio ME, Gallo V. Notch and EGFR pathway interaction regulates neural stem cell number and self-renewal. *Nature.* 2010. 467(7313): 323-7.
82. Purow BW, Sundaresan TK, Burdick MJ *et al.* Notch-1 regulates transcription of the epidermal growth factor receptor through p53. *Carcinogenesis.* 2008. 29(5): 918-25.
83. Blokzijl A, Dahlqvist C, Reissmann E *et al.* Cross-talk between the Notch and TGF-beta signaling pathways mediated by interaction of the Notch intracellular domain with Smad3. *J Cell Biol.* 2003. 163(4): 723-8.
84. Dahlqvist C, Blokzijl A, Chapman G *et al.* Functional Notch signaling is required for BMP4-induced inhibition of myogenic differentiation. *Development.* 2003. 130(24): 6089-99.
85. Takizawa T, Ochiai W, Nakashima K, Taga T. Enhanced gene activation by Notch and BMP signaling cross-talk. *Nucleic Acids Res.* 2003. 31(19): 5723-31.
86. Androutsellis-Theotokis A, Leker RR, Soldner F *et al.* Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature.* 2006. 442(7104): 823-6.
87. Huang M, Chang A, Choi M, Zhou D, Anania FA, Shin CH. Antagonistic interaction between Wnt and Notch activity modulates the regenerative capacity of a zebrafish fibrotic liver model. *Hepatology.* 2014. 60(5): 1753-66.
88. Li B, Jia Z, Wang T *et al.* Interaction of Wnt/beta-catenin and notch signaling in the early stage of cardiac differentiation of P19CL6 cells. *J Cell Biochem.* 2012. 113(2): 629-39.

89. Casso DJ, Biehs B, Kornberg TB. A novel interaction between hedgehog and Notch promotes proliferation at the anterior-posterior organizer of the *Drosophila* wing. *Genetics*. 2011. 187(2): 485-99.
90. Xie G, Karaca G, Swiderska-Syn M *et al.* Cross-talk between Notch and Hedgehog regulates hepatic stellate cell fate in mice. *Hepatology*. 2013. 58(5): 1801-13.
91. Liu S, Breit S, Danckwardt S, Muckenthaler MU, Kulozik AE. Downregulation of Notch signaling by gamma-secretase inhibition can abrogate chemotherapy-induced apoptosis in T-ALL cell lines. *Ann Hematol*. 2009. 88(7): 613-21.
92. Grabher C, von Boehmer H, Look AT. Notch 1 activation in the molecular pathogenesis of T-cell acute lymphoblastic leukaemia. *Nat Rev Cancer*. 2006. 6(5): 347-59.
93. Lobry C, Oh P, Aifantis I. Oncogenic and tumor suppressor functions of Notch in cancer: it's NOTCH what you think. *J Exp Med*. 2011. 208(10): 1931-5.
94. Weng AP, Ferrando AA, Lee W *et al.* Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science*. 2004. 306(5694): 269-71.
95. Gallahan D, Kozak C, Callahan R. A new common integration region (int-3) for mouse mammary tumor virus on mouse chromosome 17. *J Virol*. 1987. 61(1): 218-20.
96. Brennan K, Clarke RB. Combining Notch inhibition with current therapies for breast cancer treatment. *Ther Adv Med Oncol*. 2013. 5(1): 17-24.
97. Rizzo P, Miao H, D'Souza G *et al.* Cross-talk between notch and the estrogen receptor in breast cancer suggests novel therapeutic approaches. *Cancer Res*. 2008. 68(13): 5226-35.
98. Wang Z, Li Y, Banerjee S, Sarkar FH. Emerging role of Notch in stem cells and cancer. *Cancer Lett*. 2009. 279(1): 8-12.
99. Leong KG, Niessen K, Kulic I *et al.* Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. *J Exp Med*. 2007. 204(12): 2935-48.
100. Stylianou S, Clarke RB, Brennan K. Aberrant activation of notch signaling in human breast cancer. *Cancer Res*. 2006. 66(3): 1517-25.
101. Pece S, Serresi M, Santolini E *et al.* Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol*. 2004. 167(2): 215-21.
102. Abravanel DL, Belka GK, Pan TC *et al.* Notch promotes recurrence of dormant tumor cells following HER2/neu-targeted therapy. *J Clin Invest*. 2015. 125(6): 2484-96.
103. Harrison H, Farnie G, Howell SJ *et al.* Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res*. 2010. 70(2): 709-18.
104. Reedijk M, Odorcic S, Chang L *et al.* High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. *Cancer Res*. 2005. 65(18): 8530-7.
105. O'Neill CF, Urs S, Cinelli C *et al.* Notch2 signaling induces apoptosis and inhibits human MDA-MB-231 xenograft growth. *Am J Pathol*. 2007. 171(3): 1023-36.
106. Yamaguchi N, Oyama T, Ito E *et al.* NOTCH3 signaling pathway plays crucial roles in the proliferation of ErbB2-negative human breast cancer cells. *Cancer Res*. 2008. 68(6): 1881-8.
107. Reichrath J, Reichrath S. Notch-signaling and nonmelanoma skin cancer: an ancient friend, revisited. *Adv Exp Med Biol*. 2012. 727: 265-71.
108. Balint K, Xiao M, Pinnix CC *et al.* Activation of Notch1 signaling is required for beta-catenin-mediated human primary melanoma progression. *J Clin Invest*. 2005. 115(11): 3166-76.
109. Liu ZJ, Xiao M, Balint K *et al.* Notch1 signaling promotes primary melanoma progression by activating mitogen-activated protein kinase/phosphatidylinositol 3-kinase-Akt pathways and up-regulating N-cadherin expression. *Cancer Res*. 2006. 66(8): 4182-90.
110. Maillard I, Pear WS. Notch and cancer: best to avoid the ups and downs. *Cancer Cell*. 2003. 3(3): 203-5.

111. Rangarajan A, Talora C, Okuyama R *et al.* Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *EMBO J.* 2001. 20(13): 3427-36.
112. Devgan V, Mammucari C, Millar SE, Brisken C, Dotto GP. p21WAF1/Cip1 is a negative transcriptional regulator of Wnt4 expression downstream of Notch1 activation. *Genes Dev.* 2005. 19(12): 1485-95.
113. Nguyen BC, Lefort K, Mandinova A *et al.* Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. *Genes Dev.* 2006. 20(8): 1028-42.
114. Nicolas M, Wolfer A, Raj K *et al.* Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet.* 2003. 33(3): 416-21.
115. George J, Lim JS, Jang SJ *et al.* Comprehensive genomic profiles of small cell lung cancer. *Nature.* 2015. 524(7563): 47-53.
116. Wang NJ, Sanborn Z, Arnett KL *et al.* Loss-of-function mutations in Notch receptors in cutaneous and lung squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 2011. 108(43): 17761-6.
117. Sriuranpong V, Borges MW, Ravi RK *et al.* Notch signaling induces cell cycle arrest in small cell lung cancer cells. *Cancer Res.* 2001. 61(7): 3200-5.
118. Hu W, Lu C, Dong HH *et al.* Biological roles of the Delta family Notch ligand Dll4 in tumor and endothelial cells in ovarian cancer. *Cancer Res.* 2011. 71(18): 6030-9.
119. Hernandez SL, Banerjee D, Garcia A *et al.* Notch and VEGF pathways play distinct but complementary roles in tumor angiogenesis. *Vasc Cell.* 2013. 5(1): 17.
120. Dufraigne J, Funahashi Y, Kitajewski J. Notch signaling regulates tumor angiogenesis by diverse mechanisms. *Oncogene.* 2008. 27(38): 5132-7.
121. Funahashi Y, Hernandez SL, Das I *et al.* A notch1 ectodomain construct inhibits endothelial notch signaling, tumor growth, and angiogenesis. *Cancer Res.* 2008. 68(12): 4727-35.
122. Li JL, Sainson RC, Shi W *et al.* Delta-like 4 Notch ligand regulates tumor angiogenesis, improves tumor vascular function, and promotes tumor growth in vivo. *Cancer Res.* 2007. 67(23): 11244-53.
123. Noguera-Troise I, Daly C, Papadopoulos NJ *et al.* Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature.* 2006. 444(7122): 1032-7.
124. Ridgway J, Zhang G, Wu Y *et al.* Inhibition of Dll4 signalling inhibits tumour growth by deregulating angiogenesis. *Nature.* 2006. 444(7122): 1083-7.
125. Yan M, Callahan CA, Beyer JC *et al.* Chronic DLL4 blockade induces vascular neoplasms. *Nature.* 2010. 463(7282): E6-7.
126. Debeb BG, Cohen EN, Boley K *et al.* Pre-clinical studies of Notch signaling inhibitor RO4929097 in inflammatory breast cancer cells. *Breast Cancer Res Treat.* 2012. 134(2): 495-510.
127. Simmons MJ, Serra R, Hermance N, Kelliher MA. NOTCH1 inhibition in vivo results in mammary tumor regression and reduced mammary tumorsphere-forming activity in vitro. *Breast Cancer Res.* 2012. 14(5): R126.
128. Zhu TS, Costello MA, Talsma CE *et al.* Endothelial cells create a stem cell niche in glioblastoma by providing NOTCH ligands that nurture self-renewal of cancer stem-like cells. *Cancer Res.* 2011. 71(18): 6061-72.
129. Aste-Amezaga M, Zhang N, Lineberger JE *et al.* Characterization of Notch1 antibodies that inhibit signaling of both normal and mutated Notch1 receptors. *PLoS One.* 2010. 5(2): e9094.
130. Yen WC, Fischer MM, Axelrod F *et al.* Targeting Notch signaling with a Notch2/Notch3 antagonist (tarextumab) inhibits tumor growth and decreases tumor-initiating cell frequency. *Clin Cancer Res.* 2015. 21(9): 2084-95.
131. Scehnet JS, Jiang W, Kumar SR *et al.* Inhibition of Dll4-mediated signaling induces proliferation of immature vessels and results in poor tissue perfusion. *Blood.* 2007. 109(11): 4753-60.

132. Reynolds ND, Lukacs NW, Long N, Karpus WJ. Delta-like ligand 4 regulates central nervous system T cell accumulation during experimental autoimmune encephalomyelitis. *J Immunol.* 2011. 187(5): 2803-13.
133. Takebe N, Nguyen D, Yang SX. Targeting notch signaling pathway in cancer: clinical development advances and challenges. *Pharmacol Ther.* 2014. 141(2): 140-9.
134. Hayashi I, Takatori S, Urano Y *et al.* Neutralization of the gamma-secretase activity by monoclonal antibody against extracellular domain of nicastrin. *Oncogene.* 2012. 31(6): 787-98.
135. Small D, Kovalenko D, Kacer D *et al.* Soluble Jagged 1 represses the function of its transmembrane form to induce the formation of the Src-dependent chord-like phenotype. *J Biol Chem.* 2001. 276(34): 32022-30.
136. Nichol D, Shawber C, Fitch MJ *et al.* Impaired angiogenesis and altered Notch signaling in mice overexpressing endothelial Egf17. *Blood.* 2010. 116(26): 6133-43.
137. Kolb EA, Gorlick R, Keir ST *et al.* Initial testing (stage 1) by the pediatric preclinical testing program of RO4929097, a gamma-secretase inhibitor targeting notch signaling. *Pediatr Blood Cancer.* 2012. 58(5): 815-8.
138. Luistro L, He W, Smith M *et al.* Preclinical profile of a potent gamma-secretase inhibitor targeting notch signaling with in vivo efficacy and pharmacodynamic properties. *Cancer Res.* 2009. 69(19): 7672-80.
139. He W, Luistro L, Carvajal D *et al.* High tumor levels of IL6 and IL8 abrogate preclinical efficacy of the gamma-secretase inhibitor, RO4929097. *Mol Oncol.* 2011. 5(3): 292-301.
140. Huynh C, Poliseno L, Segura MF *et al.* The novel gamma secretase inhibitor RO4929097 reduces the tumor initiating potential of melanoma. *PLoS One.* 2011. 6(9): e25264.
141. Nair JS, Sheikh T, Ho AL, Schwartz GK. PTEN regulates sensitivity of melanoma cells to RO4929097, the gamma-secretase inhibitor. *Anticancer Res.* 2013. 33(4): 1307-16.
142. Saito N, Fu J, Zheng S *et al.* A high Notch pathway activation predicts response to gamma secretase inhibitors in proneural subtype of glioma tumor-initiating cells. *Stem Cells.* 2014. 32(1): 301-12.
143. Dantas-Barbosa C, Bergthold G, Daudigeos-Dubus E *et al.* Inhibition of the NOTCH pathway using gamma-secretase inhibitor RO4929097 has limited antitumor activity in established glial tumors. *Anticancer Drugs.* 2015. 26(3): 272-83.
144. LoConte NK, Razak AR, Ivy P *et al.* A multicenter phase 1 study of gamma -secretase inhibitor RO4929097 in combination with capecitabine in refractory solid tumors. *Invest New Drugs.* 2015. 33(1): 169-76.
145. Wei P, Walls M, Qiu M *et al.* Evaluation of selective gamma-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. *Mol Cancer Ther.* 2010. 9(6): 1618-28.
146. Lopez-Guerra M, Xargay-Torrent S, Rosich L *et al.* The gamma-secretase inhibitor PF-03084014 combined with fludarabine antagonizes migration, invasion and angiogenesis in NOTCH1-mutated CLL cells. *Leukemia.* 2015. 29(1): 96-106.
147. Samon JB, Castillo-Martin M, Hadler M *et al.* Preclinical analysis of the gamma-secretase inhibitor PF-03084014 in combination with glucocorticoids in T-cell acute lymphoblastic leukemia. *Mol Cancer Ther.* 2012. 11(7): 1565-75.
148. Zhang CC, Yan Z, Zong Q *et al.* Synergistic effect of the gamma-secretase inhibitor PF-03084014 and docetaxel in breast cancer models. *Stem Cells Transl Med.* 2013. 2(3): 233-42.
149. Grottkau BE, Chen XR, Friedrich CC *et al.* DAPT enhances the apoptosis of human tongue carcinoma cells. *Int J Oral Sci.* 2009. 1(2): 81-9.
150. Jing W, Xiong Z, Cai X *et al.* Effects of gamma-secretase inhibition on the proliferation and vitamin D(3) induced osteogenesis in adipose derived stem cells. *Biochem Biophys Res Commun.* 2010. 392(3): 442-7.

151. Mori M, Miyamoto T, Yakushiji H *et al.* Effects of N-[N-(3, 5-difluorophenacetyl-L-alanyl)]-S-phenylglycine t-butyl ester (DAPT) on cell proliferation and apoptosis in Ishikawa endometrial cancer cells. *Hum Cell.* 2012. 25(1): 9-15.
152. Li LC, Peng Y, Liu YM, Wang LL, Wu XL. Gastric cancer cell growth and epithelial-mesenchymal transition are inhibited by gamma-secretase inhibitor DAPT. *Oncol Lett.* 2014. 7(6): 2160-2164.
153. Wang M, Ma X, Wang J, Wang L, Wang Y. Pretreatment with the gamma-secretase inhibitor DAPT sensitizes drug-resistant ovarian cancer cells to cisplatin by downregulation of Notch signaling. *Int J Oncol.* 2014. 44(4): 1401-9.
154. Khazaei M, Kalantari E, Saeidi H *et al.* Gamma-secretase inhibitor does not modulate angiogenesis in colon adenocarcinoma in obese mice. *Bratisl Lek Listy.* 2015. 116(4): 248-51.
155. Tammam J, Ware C, Efferson C *et al.* Down-regulation of the Notch pathway mediated by a gamma-secretase inhibitor induces anti-tumour effects in mouse models of T-cell leukaemia. *Br J Pharmacol.* 2009. 158(5): 1183-95.
156. Pandya K, Meeke K, Clementz AG *et al.* Targeting both Notch and ErbB-2 signalling pathways is required for prevention of ErbB-2-positive breast tumour recurrence. *Br J Cancer.* 2011. 105(6): 796-806.
157. Ramakrishnan V, Ansell S, Haug J *et al.* MRK003, a gamma-secretase inhibitor exhibits promising in vitro pre-clinical activity in multiple myeloma and non-Hodgkin's lymphoma. *Leukemia.* 2012. 26(2): 340-8.
158. Plentz R, Park JS, Rhim AD *et al.* Inhibition of gamma-secretase activity inhibits tumor progression in a mouse model of pancreatic ductal adenocarcinoma. *Gastroenterology.* 2009. 136(5): 1741-9 e6.
159. Timme CR, Gruidl M, Yeatman TJ. Gamma-secretase inhibition attenuates oxaliplatin-induced apoptosis through increased Mcl-1 and/or Bcl-xL in human colon cancer cells. *Apoptosis.* 2013. 18(10): 1163-74.
160. De Keersmaecker K, Lahortiga I, Mentens N *et al.* In vitro validation of gamma-secretase inhibitors alone or in combination with other anti-cancer drugs for the treatment of T-cell acute lymphoblastic leukemia. *Haematologica.* 2008. 93(4): 533-42.
161. Meng X, Matlawska-Wasowska K, Girodon F *et al.* GSI-I (Z-LLNle-CHO) inhibits gamma-secretase and the proteasome to trigger cell death in precursor-B acute lymphoblastic leukemia. *Leukemia.* 2011. 25(7): 1135-46.
162. Schott AF, Landis MD, Dontu G *et al.* Preclinical and clinical studies of gamma secretase inhibitors with docetaxel on human breast tumors. *Clin Cancer Res.* 2013. 19(6): 1512-24.
163. Albain K, Czerlanis C, Zlobin A *et al.* Modulation of cancer and stem cell biomarkers by the notch inhibitor MK-0725 added to endocrine therapy for early stage ER+ breast cancer. *Cancer Research.* 2011. 71(24 Supplement): S1-5.
164. Krop I, Demuth T, Guthrie T *et al.* Phase I pharmacologic and pharmacodynamic study of the gamma secretase (Notch) inhibitor MK-0752 in adult patients with advanced solid tumors. *J Clin Oncol.* 2012. 30(19): 2307-13.
165. Wu Y, Cain-Hom C, Choy L *et al.* Therapeutic antibody targeting of individual Notch receptors. *Nature.* 2010. 464(7291): 1052-7.
166. Moellering RE, Cornejo M, Davis TN *et al.* Direct inhibition of the NOTCH transcription factor complex. *Nature.* 2009. 462(7270): 182-8.
167. Epenetos A, Kosparou C, Stylianou S. Inhibition of Notch signaling for the treatment of human carcinomas. *Proceedings of the American Association of Cancer Research.* 2009. 18-22 April, Denver CO.