INVITED REVIEW

Targeting the Notch signaling pathway in cancer therapeutics

Huajiao Guo[1,2], Yi Lu[1,2], Jianhua Wang[3,4], Xia Liu[1,2], Evan T. Keller[5], Qian Liu[6], Qinghua Zhou[6] & Jian Zhang[1,2,5]

1 Key Laboratory of Longevity and Aging-Related Diseases, Ministry of Education, Nanning, China
2 Center for Translational Medicine, Guangxi Medical University, Nanning, China
3 Department of Biochemistry and Molecular & Cell Biology, Key Laboratory of Cell Differentiation and Apoptosis, Ministry of Education, Shanghai, China
4 Institute of Medical Science, Shanghai JiaoTong University School of Medicine, Shanghai, China
5 Department of Urology and Pathology, School of Medicine, University of Michigan, Ann Arbor, Michigan, USA
6 Tianjin Key Laboratory of Lung Cancer Metastasis and Tumor Microenvironment, Tianjin Lung Cancer Institute, Tianjin Medical University General Hospital, Tianjin, China

Keywords
Cancer; Notch signaling pathway; oncogene; targeted therapeutics; tumor suppressor gene.

Correspondence
Jian Zhang or Qinghua Zhou, Guangxi Medical University, Room 1418, Pharmacology and Biomedical Sciences Building, no. 22 Shuangyong Road, Nanning, 530021, China.
Tel: +86 771 5300270
Fax: +86 771 5310865
Email: jzhangqi@gmail.com; zhouqh1016@yahoo.com.cn

Received: 28 April 2014;
Accepted: 18 May 2014.

doi: 10.1111/1759-7714.12143

Thoracic Cancer **5** (2014) 473–486

Abstract

Despite advances in surgery, imaging, chemotherapy, and radiotherapy, the poor overall cancer-related death rate remains unacceptable. Novel therapeutic strategies are desperately needed. Nowadays, targeted therapy has become the most promising therapy and a welcome asset to the cancer therapeutic arena. There is a large body of evidence demonstrating that the Notch signaling pathway is critically involved in the pathobiology of a variety of malignancies. In this review, we provide an overview of emerging data, highlight the mechanism of the Notch signaling pathway in the development of a wide range of cancers, and summarize recent progress in therapeutic targeting of the Notch signaling pathway.

Introduction

The Notch signaling pathway, a highly evolutionarily conserved pathway in both invertebrate and vertebrate development, plays a key role in cell differentiation, survival, and proliferation. In 1917, Morgan's group first described Notch mutant Drosophila, in which the ends of the wings were characterized by a serration.[1] Half of the sons of heterozygous female flies with Notch mutant wings suffered from embryonic period death as a result of multiple defects. This suggests that notch signaling is indispensable during development.

Notch receptors and ligands

In 1980s, the Notch gene was first cloned by Artavanis-Tsakonas *et al.* and identified as a locus affecting neurogenesis.[2] Subsequent studies showed that Notch families have four receptors (Notch1-4) in mammals, which are type I transmembrane proteins (one kind of protein which anchor to the cell membrane with an anchor sequence and have their N-terminal domains targeted to the ER lumen during synthesis).[3,4] Each Notch receptor is synthesized as a full-length precursor protein (300–350 kDa), consisting of a Notch extracellular domain (NECD), a transmembrane domain, and an intracellular domain. All four Notch receptors are similar except for subtle differences in their extracellular and cytoplasmic domains. The extracellular domain contains ~30 epidermal growth factor (EGF)-like repeats that participate in ligand-binding followed by a conserved negative regulatory region (NRR or LNR) consisting of three LIN repeats (Lin-12/Notch repeats) and a heterodimerization region that is involved in activation of Notch signaling while binding to the relevant ligands. Notch family members differ in the number of EGF-like repeats, both Notch-1 and Notch-2 proteins have 36 arranged repeats of EGF-like domain, whereas Notch-3 and Notch-4 contain 34 and 29 EGF-like repeats,

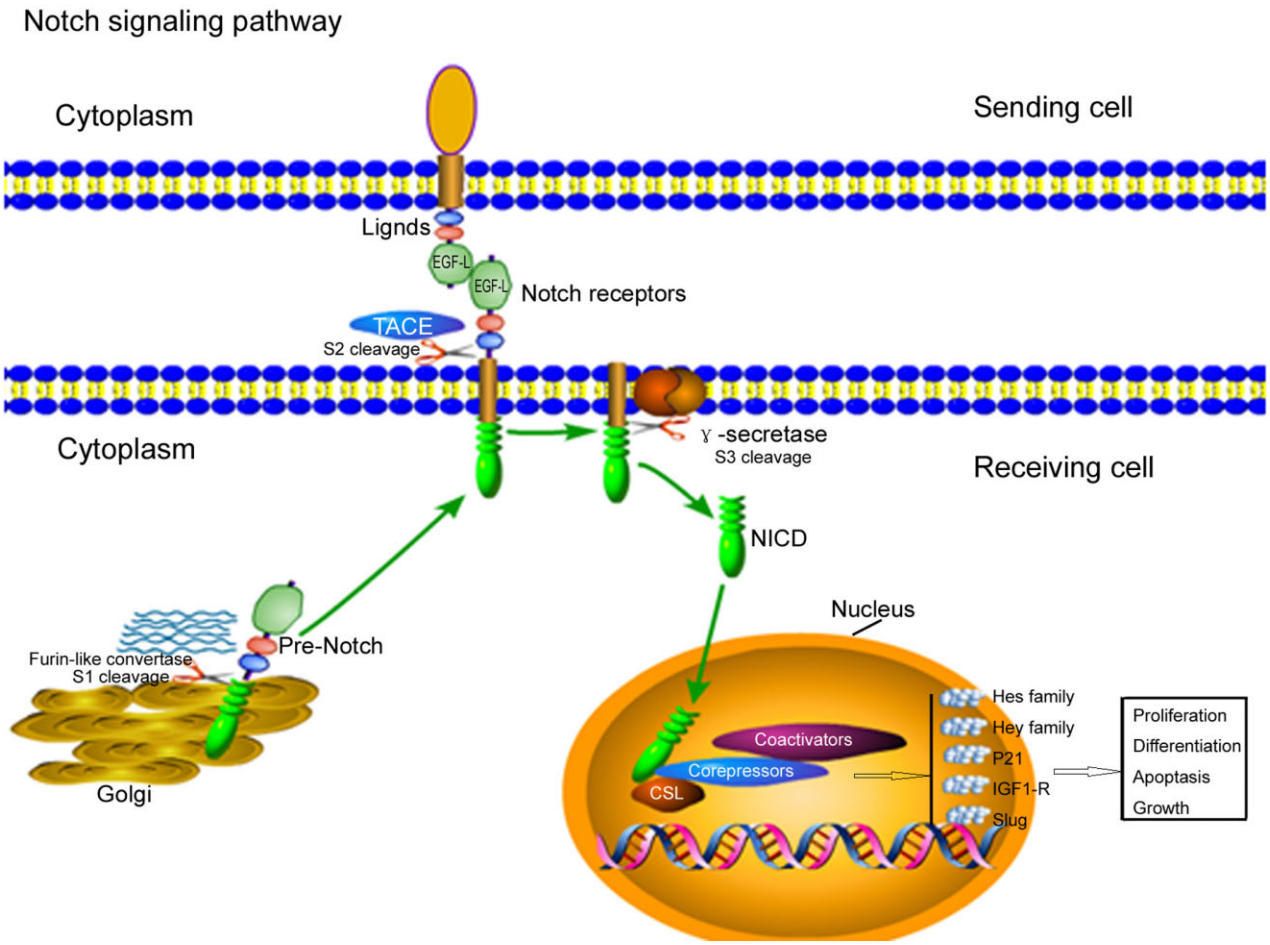

Figure 1 Activation of the Notch signaling pathway.

respectively.[5] EGF-like repeats mediate ligand binding, whereas NRR functions to prevent both ligand-dependent and –independent signaling.[6] The Notch intracellular domains (NICD) contain a regulation of amino-acid metabolism (RAM) domain (high affinity CSL [CBF1, Suppressor of Hairless, Lag-1] binding site, binding to the downstream target genes), six ankryin repeats (cell division cycle gene 10, flanked by nuclear localization signals and regulating the transfection of downstream genes), nuclear localization signals (NLS), and a carboxy-terminal praline-glutamate-serine-threonine (PEST rich region, involved in the deregulation of Notch proteins) sequence.[7] Five DSL (named for Delta and Serrate from Drosophila and Lag-2 from *C. elegans*) ligands Jagged1, Jagged2, delta-like 1 (DLL1), DLL3, and DLL4 have been described in mammals. Similar to Notch receptors, Notch ligands also contain a set of EGF-like repeats in their extracellular domain, a DSL domain, and a cysteine-rich region (CR) in Serrate, which are absent in Delta. Jagged1 and Jagged2 have almost two-fold numbers of EGF-like repeats compared to Delta.[8] The DSL domain is highly conserved in Ligands families and is essential for Notch activity. Notch ligands are also transmembrane proteins, while the intracellular domains only contain 70~215 amino acid residues.[9]

Notch activation

Seven key signal transduction pathways that control cell communication during animal development have been identified: Wnt, transforming growth factor-β (TGF-β), Hedgehog (Hh), receptor tyrosine kinase (RTK), nuclear receptor, Jak/STAT, and Notch signaling. Intriguingly, Notch is the only pathway that relies on cell-cell contact.[10,11] The activation of Notch signaling mainly contains three proteolytic events (Fig. 1). The first cleavage is S1 cleavage: within the Golgi apparatus, furin-like convertase, the precursor proteins of Notch receptors are cleaved into two associated peptides extracellular Notch subunit (ECN) and Notch transmembrane subunit (NTM), and then the two fragments are reassembled as a non-covalently linked heterodimeric receptor at

the cell surface.[12,13] The second cleavage occurs near the extracellular side of the plasma membrane. Ligand binding triggers S2 cleavage by the tumor necrosis factor-alpha-converting enzyme (TACE), a disintegrin and metalloprotease (ADAM),[14] creating a short-lived membrane-bound intermediate lacking most of the Notch ectodomain that is a substrate for γ-secretase, a multisubunit intramembranous protease. The γ-Secretase-mediated S3 cleavage occurs on plasma membrane and in endosome, resulting in the release of NICD into the cytoplasm, where NICD translocates into the nucleus.[15,16] Once in the nucleus, the NICD forms a complex by binding to the ubiquitously expressed transcription factor CSL[17] via its RAM and ankryin domains. In the absence of NICD, CSL functions as a transcriptional repressor because it interplays with ubiquitous corepressor (Co-R) proteins and histone deacetylases (HDACs) to repress transcription of some target genes.[18–22] With the binding of NICD, the CSL family is converted in to a transcriptional activator by displacing corepressors (e.g. MTG8, MTG16, and SPEN) and by recruiting coactivators, such as Mastermind, and the histone acetyltransferase, to activate transcription of Notch target genes.[23–25]

Notch signaling target genes

The events of activation of the Notch signaling pathway result in transcription of target genes. The well-known direct target genes of CSL are transcriptional repressors, such as Hes (Drosophila genes hairy and Enhancer of split [Hes1-7]) and Hey subfamilies (Hey1, Hey2, HeyL, HesL/HelT, Dec1/BHLHB2, Dec2/BHLHB3).[26–28] Both Hes and Hey subfamilies contain a basic domain, which determines DNA binding specificity, and a basic-helix-loop-helix domain (bHLH), which allows proteins to form homo- or heterodimers.[29] The Hes bHLH repressor genes play an essential role in the development of many organs by maintaining progenitor cells and regulating binary cell fate decisions. In these processes, Hes genes (Hes1 and Hes5) function as effectors of Notch signaling, which coordinate cellular events via cell-cell interactions.[30] In the absence of Hes1 and Hes5, the NICD cannot inhibit neurogenesis, indicating that Hes1 and Hes5 are essential effectors of Notch signaling in the nervous system.[19,31] In addition to particular differentiation-related factors, the transcriptional targets of Notch signaling also include proteins and factors involved in the control of cell cycle and survival processes, such as in cell cycle regulators. For example, p21 (a cyclin-dependent kinase inhibitor that acts as both a sensor and an effector of multiple anti-proliferative signals) and cyclin D1 (a mitogenic sensor and allosteric activator of cyclin-dependent kinase CDK4/6), transcription factors, such as c-MYC (an oncogene and cell cycle regulator, one of the hallmarks of many cancers) and nuclear factor kappa B (NF-κB) (a transcriptional factor), growth factor receptors such as HER/ErbB genes, regulators of apoptosis survivin (a member of the inhibitor of apoptosis family of proteins),[32–44] insulin-like growth factor 1 receptor (IGF1-R),[45] and Slug.[46]

Non-canonical Notch signaling pathway

Canonical Notch signaling (CSL-dependent signaling), is involved in many physiological and pathological events in all animals, and most of its functions and structures have been reported. But the knowledge of non-canonical Notch signaling is relatively inadequate. The definition of the non-canonical signals is broad and mainly contains the following: DSL-independent activations, interactions with non-DSL ligands, CSL-independent signaling, signal transduction without cleavage, differential posttranslational modifications, and competition/protection for a cofactor.[47,48] Though the core pathway plays a key role in the development of lives, the role of non-canonical Notch signaling in many biological events has drawn increasing attention. In the last decade, a growing number of studies have reported that non-canonical Notch signaling affects the occurrence and development of tumors and other disorders in the manner of interacting with other signalings.[49–51] These effects have not been explored, and the physiological functions of the non-canonical Notch pathway remain unclear. Studies by Jin *et al.*[49] have shown that the non-canonical Notch pathway contributed to the tumor process by up-regulating interleukin (IL)-6 in both basal breast clinical specimens and cancer cells. It has been demonstrated that Notch acts as an endogenous immune regulator, which moderates cytokine expression of dendritic cells through the non-canonical Notch pathway.[50]

Cross-talk among Notch pathway and other pathways

Notch plays critical roles in both invertebrate and vertebrate development and nearly correlates with the process of forming and developing many diseases, especially tumors, largely through its interaction with other signaling pathways, such as developmental signals, growth factors, and inflammatory cytokines, as well as transcriptional factors. It has been summarized by several reviews.[52–55] In this review, we will summarize the cross-talk among Notch and other pathways, such as Wnt, IL-6, and the urokinase-type plasminogen activator (uPA)/urokinase plasminogen activator receptor (uPAR) axis in tumor progression.

Wnt signaling pathway

Similar to Notch signaling, Wnts were first discovered in Drosophila. The Wnt signaling pathway (named as a hybrid of Wingless and Int) is highly conserved in mammals and plays a crucial role in the development of tissues and organisms.[56]

Three different Wnt-regulated pathways: the canonical Wnt/β-catenin and two non-canonical pathways (planar cell polarity pathway, Wnt/Ca2 + pathway) have been identified. Canonical Wnt signals are transduced through Frizzled family receptors and LRP5/LRP6 coreceptors to the β-catenin signaling cascade. Increasing evidence demonstrates that dysregulation of Wnts signaling is involved in carcinogenesis and tumorigenesis, especially in the intestine.[57–59] Wnts are also critical in bone metastasis of multiple cancers, such as multiple myeloma, prostate and breast cancers.[60]

Both Notch signaling and Wnt signaling are developmental signaling pathways, and the cross-talk between them are very common in biological events. LEF1, a transcription factor of the TCF/LEF family, which participates in the tWnt signaling pathway, was found to bind multiple sites in DLL1 promoter in vertebrate somitogenesis.[61] Among Notch ligand genes, the Jagged1 gene also was predicted as an evolutionarily conserved target of the canonical Wnt signaling pathway, based on the conservation of double TCF/LEF–binding sites within the 5′ promoter region of mammalian Jagged1 orthologues.[59,62–64] Ayyanan *et* al. demonstrated that the activation of Wnt signaling can significantly increase the expression of Notch receptors (Notch3, Notch4) and Notch target genes (Hes1, Hes5, RBP-JK), and in addition to Notch ligand genes, these events would result in the triggering of oncogenic conversion of human breast epithelial cells.[65] These observations suggest that the Wnt signal is mechanistically epistatic to the Notch signal. Therefore, the mode of cooperation might be convergent up-regulation of a common target. However, a study of loss and gain-of-function mutation of LNX2 (involved in regulating Notch signaling) in colorectal cancers (CRC) has provided convincing evidence to support an aberrant Notch-Wnt axis in CRC.[66] These findings outline a positive feedback-signaling axis by which Wnt signaling regulates Notch signaling to promote tumor proliferation. Intriguingly, an antagonistic effect could also be found in the cross-talk between Notch and Wnt signaling. On osteoblastogenesis, Notch overexpression decreased the transactivating effect of Wnt 3a, cytoplasmic β-catenin levels, and Wnt-dependent gene expression by up-regulating the expression of Hes1.[67] Likewise, Galceran *et al*. demonstrated that LEF1 binds to the DLL1 promoter sites, which regulates the somitogenesis in vertebrate.[61] Another experiment conducted by Phng *et al*.,[68] however, resulted in the opposite effect where DLL4/Notch-induced expression of Notch-regulated ankyrin repeat protein (Nrarp) limits Notch signaling and promotes Wnt/β-catenin signaling in endothelial stalk cells through interactions with LEF1. The molecular mechanisms of these different results are still unclear, which may because of the context-dependent event. Notch signaling is indispensable to the formation of the segments, but may not be critical to the formation of other organs, while Wnt signaling molecules play key roles during embryogenesis, tissue regeneration, and carcinogenesis.[55] As another line of evidence, activation of Wnt/β-catenin and inhibition of Notch signaling pathways efficiently induce intestinal differentiation of embryonic stem cells.[69] Further studies will be necessary to unravel the molecular mechanism underlying the effect of cross-talk between Notch and Wnt signaling.

Interleukin (IL)-6

Inflammatory microenvironment signaling has been shown to play a crucial role in cancer progression (i.e. cancer cell proliferation, survival, angiogenesis, and metastasis) in many types of human malignancies. As a pleiotropic and pro-inflammatory cytokine, IL-6 is important for immune responses, cell survival, apoptosis, and proliferation.[70,71] It can be produced by various types of cells, including T cells, macrophages, fibroblasts, and vascular endothelial cells. IL-6 activates IL-6 receptor (IL-6R) to initiate signaling through the Janus kinase (JAK)/signal transducers and activators of transcription (STAT) signaling pathway.[72] Accumulating evidence implicates IL-6 and its major effector STAT3 as protumorigenic agents found in cancers, including breast, lung, prostate, and hematological cancers and melanoma. Elevated levels of IL-6 correlate with poor prognosis for breast cancer patients[73–76] because elevated levels promote chemoresistance by expanding the cancer stem cell population.[77,78] Sansone *et al*. have proven that the Notch pathway is a critical downstream target of IL-6 and this is the first time a relationship between IL-6 and Notch signaling has been described.[79] Administration of anti-IL-6 yielded down-regulation in the level of Notch-3 gene expression and administration of IL-6 elicited up-regulation of Notch-3 mRNA. Similar to Sansone *et al*.'s result, Sethi *et al*.[74] confirmed that IL-6 secreted by osteoblasts may potentially stimulate tumor growth, which was significantly up-regulated with the stimulation of Jagged1. Conversely, co-culture of MC3T3-E1 cells with tumor cells, recombinant IL-6 significantly enhanced the tumor cell proliferation. Moreover, after treatment with γ-secretase inhibitor MRK-003, the transcription and secretion of IL-6 from osteoblasts were remarkably reduced. Wongchana and Palaga's[80] results demonstrated that in macrophages, the up-regulation of Notch1 increases the IL-6 gene expression, which can be blocked by treatment with γ-secretase inhibitor, which is consistent with Sethi *et al*.'s findings. More recently, the most intriguing observation made by Jin *et al*.[49] suggested that IL-6 expression is regulated by non-canonical, CSL-independent, Notch signaling. The observed up-regulation of IL-6 expression by Notch led to the autocrine and paracrine activation of JAK/STAT signaling. These findings outline a positive feedback-signaling axis by which Notch signaling stimulates the release of IL-6 to promote cancer cell proliferation. The molecular mechanism

of the Notch-IL-6 axis is probably a result of the genetic structure site. Within the IL-6 gene promoter region, the signature binding motif of CSL, a key DNA-binding protein in the Notch signaling pathway, was identified and found to overlap with a consensus NF-κB-binding site.[80] That might provide a reliable analysis for the reason why in IL-6 over-expressing tumors, γ-secretase inhibitor-RO4929097 no longer impacts angiogenesis or the infiltration of tumor associated fibroblasts.[81] A high level of IL-6 may bind to the key DNA-binding protein in the Notch signaling pathway and promote biological effects, which abrogate the preclinical efficacy of the γ-secretase inhibitor. Further understanding of the molecular nature will allow avoidance of adverse effects during possible clinical treatments.

uPA/uPAR axis

The uPA system is composed of uPA, its glycolipid (glycosylphosphatidylinositol) uPAR, plasminogen, and plasminogen activator inhibitors (PAI-1 and PAI-2). The plasminogen activator system is implicated in multiple physiological and pathologic processes including cell migration, angiogenesis, embryogenesis, tumor growth, and metastasis. The binding of uPA and its receptor uPAR catalyzes the inactive plasminogen to the active plasmin,[82,83] which would lead directly or indirectly to epithelial-mesenchymal transition (EMT), degradation of the basement membrane and release of active metalloproteinases (MMPs) that result in metastasis.[84,85] Striking experimental data suggest that uPA and uPAR are over-expressed in diverse human malignant tumors including breast,[86–88] pancreatic,[89] lung,[90,91] and andand prostate cancers[92,93] and are associated with poor patient survival,[94–96] suggesting that the uPA/uPAR axis is a cancer therapeutic target. The metastasis of a primary tumor to distant organs must undergo a multistage process that includes local cell invasion through the degradation of extracellular cell matrix (ECM) components, intravasation into the bloodstream, extravasation from the circulation, and colonization in a distant organ.[97] The uPA/uPAR axis plays a critical role in cancer metastasis, not only in its role in degrading EMT, but also regulating cell migration as a signal transduction molecule through interacting with other signals. In glioblasoma cells, down-regulating uPA/uPAR abolished *in vitro* invasion and *in vivo* tumor development by suppressing Notch1-pertinent gene expression and signaling events, resulting in the deduction of phosphorylation of protein kinase B (AKT)/extracellular signal-regulated kinases (ERK) and NF-κB.[98] Similarly, studies of several cancer types demonstrated that the down-regulation of Notch-1 or Jagged-1 led to the decreased expression and diminished bioactivity of uPA, which contributed to the inhibition of cancer cell migration, invasion, and apoptosis.[86,99,100] These studies implicate that uPA/uPAR could be the potential functional link between Notch signaling and tumor metastasis.

Notch in cancer

A role for Notch signaling in cancer was originally suggested because a chromosomal translocation that was found in a patient with T cell acute lymphoblastic leukaemia (T-ALL),[101] which opened the door to an ever-widening understanding of tumor growth controlled or influenced by Notch signaling. Notch has been shown to promote or limit tumor growth, which is highly dependent on signal dose, Notch homolog, and context.[41] Accumulating data have demonstrated that Notch signaling is a more complex process than originally thought. Here we provide a brief overview on the roles of the Notch signaling pathway in the progression of a wide range of cancers.

Notch as an oncogene

The first data which described Notch signaling as a cancer promoting factor was derived from human acute lymphoblastic leukaemia (T-ALL), a neoplastic disorder accounting for ~10–20% of all T-ALL. In 1991, Ellisen *et al.*[101] first identified a recurrent translocation t [7;9][q34;q34.4] in T-ALL patients. The translocation fused the 3′ portion of Notch1 to the T cell receptor β promoter/enhancer, resulting in the constitutively active and over-expression of an active form of Notch1 protein (N1ICD). In this seminal discovery, however, it appeared that <1% of T-ALL cases contained this translocation, thus it didn't illustrate the causal role for Notch in T-ALL carcinogenesis. More compelling evidence of the central role of Notch1 in human T-ALL was discovered by Weng *et al.*,[6] when they reported that of two types of activating mutations of Notch1 – the extracellular heterodimerization domain (that induces ligand-independent activation) and the C-terminal PEST domain (that increases the stability of N1ICD)[102] – at least one of which can be examined in more than 50% of human T-ALL. Consistent with these results, Reschly *et al.*[103] demonstrated that T cell lymphomas accumulate mutations in or near the PEST domain, which mediates degradation of the active form of Notch1. These data, however, do not classify whether these mutations are initiating or collaborating secondary events and further studies will be necessary to unravel the molecular mechanism. Further evidence for Notch signaling as an oncogene may lie in that Notch1 regulates the expression of c-MYC, a potent driver of cell cycle entry, contributing to cell cycle progression in T-ALL.[34] Sharma *et al.*[104] demonstrated that Notch1 directly induces the expression of c-MYC and that inhibition of Notch1 using small molecule inhibitors of the γ-secretase complex resulted in cell cycle arrest and apoptosis and decreased c-MYC levels. These studies and those

performed by Palomero *et al*.[105] in human T-ALL cell lines, which elegantly showed that the interaction of Notch1 and c-MYC was composed of a feed-forward-loop regulatory motif controlling leukemic cell growth, demonstrate that this interaction of Notch1 and c-MYC is required to maintain the growth. Beverly *et al*.[106] suggested that Notch1 also suppressed p53 function in T-ALL cells, which could promote oncogenesis through increased cell survival and genomic instability. Collectively, these studies delineate how Notch1 mediated cellular transformation in human T-ALL and provide a more reliable theoretical basis for the treatment of T-ALL.

After the discovery of its involvement in T-ALL, Notch signaling was also implicated in breast cancer.[107–110] The oncogenic potential of Notch activation in solid tumors was first observed in murine mammary cancer,[111] which is induced by the mouse mammary tumor virus (MMTV). The MMTV induced a mammary tumor by insertion into the genome and deregulating expression of adjacent integration (int3) genes, later identified as the Notch4 locus.[112] A substantial body of evidence in subsequent decades, derived not only from preclinical, but also clinical studies, has accumulated in support of Notch signaling playing important oncogenic roles in human breast cancer as well. Breast cancer patients with high levels of Notch1 and Jagged1 showed a poorer prognostic profile and lower survival rates.[110,113] Similarly, one study has shown that more than 50% of human breast tumors express reduced protein levels of Numb, a negative regulator of Notch signaling, which has been associated with high-grade breast cancers.[114] As in T-ALL, c-MYC is a direct downstream effector of Notch1, and co-expression of Notch1 and c-MYC has been found in a large fraction of examined human breast cancers by comparative expression profile analysis using microarrays.[42] Furthermore, the most intriguing observation made by Sethi *et al*.[74] indicated that tumor-derived Jagged1 promoted the bone metastasis of breast cancer by stimulating IL-6 release, which can be reversed after treatment with γ-secretase inhibitor. Taken together, these findings suggest that aberrant Notch signaling may be of great importance in human breast cancer and provide a rationale for targing Notch signaling in breast cancer.

The expression of Notch receptors and their downstream target genes is also up-regulated in primary human pancreatic,[115,116] lung,[117] and liver cancers.[118] The enforced expression of constitutively active Notch also promotes melanoma progression.[119,120] Because it plays a key role in three tumor survival processes: tumor cell transformation, survival, and angiogenesis,[121,122] the Notch axis is often considered as oncogenic. In addition, although underlying mechanisms are yet to be fully elucidated, Notch has been shown to facilitate epithelial-mesenchymal transition[123,124] and induce cancer stem cell-like properties in breast cancer cells.[108,125] Although this is true in some cases, it certainly does not represent all tumor types.

Notch as tumor suppressor

Although Notch was originally identified as an oncogene, studies have also demonstrated that components of the same pathway may have growth-suppressive functions in some hematopoietic cells, skin, and pancreatic epithelium, as well as in hepatocytes, illustrating the highly context-dependent nature of the pathway. The first evidence describing Notch signaling as a factor for suppressing tumors was derived from Nicolas *et al*. In their study, mice with Notch1-deficient epithelia increased and sustained expression of Gli2, which is a downstream component of the Sonic–hedgehog (SHH)-signaling pathway, causing the development of spontaneous basal-cell-carcinoma-like tumors over time.[126] Consistent with this, Thelu *et al*.[127] reported that expressions of Notch1, Notch2, and Jagged1 were down-regulated in human basal-cell carcinomas. These results indicated that a loss of Notch signaling in human epidermis, as well as in mouse epithelia, could lead to the development of basal-cell carcinomas through suppression of the SHH pathways. Another Notch target gene that appears to contribute tumor suppressive effects in the epidermis is β-catenin, a regulator of Wnt signaling. Researchers have demonstrated that activation of Wnt signaling is observed in basal-cell-carcinoma-like tumors,[128,129] however, these results do not illustrate the causal role for Wnt signaling in these tumors. Instead, they highlight the ability of the Notch pathway in Notch1-deficient mouse skin where the Wnt pathway is re-activated resulting in increased β-catenin-mediated signalling, which generates these cancers.[126] Furthermore, Wnt4 was found to be negatively regulated by Notch in mouse keratinocytes and skin through $p21^{WAF1/Cip1}$, a negative transcriptional regulator of Wnt4 expression.[130] Thus, mechanistically, tumor inhibition in the skin may involve feedback with the microenvironment in addition to cross-talk between Notch and other signaling pathways.

The studies on Notch function in skin lead to an interesting question: Is the tumor suppressive activity of Notch manifested in a broader range of tissues? Evidence from several studies on Notch function in neuroendocrine tumors (NETs), such as small-cell lung cancer (SCLC), pancreatic carcinoid, and medullary thyroid cancer (MTC), seem to support this notion.[32,131–134] In non-small cell lung cancer (NSCLC), Notch shows a growth promoting function,[117,135–137] whereas in SCLC it exerts an inhibitory effect.[134,138] These apparent but paradoxical functions clearly indicate that the role of Notch signaling is dependent on its cellular context. In SCLC, constitutively active Notch receptors (Notch1, Notch2) have been shown to cause a profound growth arrest,[134] which may be associated with a G1 cell cycle block through up-regulating the expression of $p21^{waf1/Cip1}$ and $p27^{Kip1}$.[139] In Sriuranpong *et al*.'s study, they also illuminated another possible mechanism of Notch as a tumor suppressor,

the constitutive activation of Notch proteins led to the reduction of human achaete-scute homologue-1 (hASH1) expression, a transcription factor of basic-helix-loop-helix (bHLH), as well as the activation of phosphorylated ERK1 and ERK2, resulting in cell cycle arrest in SCLC cells. Similarly, the deletion of Notch1 accelerates PanIN development[140] and increases the incidence and progression of pancreatic ductal adenocarcinoma (PDAC), which is induced by the most commonly mutated oncogene K-ras,[141] implying that *Notch1* can function as a tumor suppressor gene in PDAC. Viatour *et al.*[142] showed that the expression of Notch receptors (Notch1-4), as well as a downstream transcriptional target of the Notch pathway, was higher in TKO hepatocellular carcinoma (HCC) models than that in other HCC mouse models, which raised the question that Notch signaling in the liver might act as an oncogene. Unexpectedly, inhibition of Notch signaling with DAPT in TKO mice increased the development of cancer, and liver-specific inactivation of Notch1 expression also leads to the proliferation of hepatocytes in mice.[143] In contrast, increased Notch activity leads to cell cycle arrest in G2 and/or cell death in TKO fibroblasts,[144] as well as in human HCC cell lines.[145] The discrepancies in these studies may result from loss-of-function versus gain-of-function approaches, as well as differences in the model systems. To address the relevance of these observations, Viatour *et al.*[142] found that liver cancer patients with significantly higher expression of Notch1 and Hes1 could survive longer, showing TKO dataset preferentially. Viatour *et al.*'s study also indicates that Notch signaling acts as a tumor suppressor feedback mechanism in response to activation of E2F transcription factors in TKO liver cells. Finally, Notch1 also seems to be a $p53^{bv}$ target gene, which negatively regulates Rho GTPase effector genes, thereby decreasing cell adhesion and stimulating terminal differentiation.[146–149] However, the growth inhibitory role of Notch has been mainly suggested on the basis of activated Notch1 overexpression studies. Thus, the extent and frequency of Notch to inhibit the proliferation of cancer must still be verified.

Notch in cancer stem cells

Cancer stem cells (CSCs, also known as tumor-initiating cells) are rare cells with indefinite potential for self-renewal that drive tumorigenesis.[150] CSCs are an attractive candidate as the origin of cancer, and may be responsible for the relapse and metastasis of tumors, which are still major obstacles for improving overall cancer survival. Bonnet and Dick's studies first described cancer stem cells,[151] specifically that human acute myeloid leukaemia (AML) cells originate from a primitive hematopoietic cell, which shows cell surface markers $CD34^{+}$ $CD38^{-}$, exclusively, and target for leukaemia transformation. A number of studies have also identified CSCs in many solid tumors, such as prostate,[152–154] pancreatic,[155] colon,[156,157] liver,[158] lung, and breast cancers.[160]

As cancer stem cells behave like normal stem cells, it is believed that genetic alterations in critical signaling pathways that govern stem cells would also play important roles in cancer stem cells. These pathways include: Notch, Wnt, bone morphogenic protein (BMP), and Sonic hedgehog signaling pathways.[161–164] The deregulation of these pathways, resulting in stem cell expansion, may be a key event originating CSCs and, thereby, initiating carcinogenesis. But the causal relationship between Notch signaling and CSCs is not clear. Gain-of-function of Notch studies in mouse mammary stem cells (MaSCs) suggest that inappropriate Notch activation contributes to mammary carcinogenesis by promoting the self-renewal and transformation of luminal progenitor cells.[165] Similarly, experiments on human breast cancer stem cells, which were enriched using cell surface markers, such as CD44+/CD24−, showed an upregulation of Notch gene expression. Contrary to this finding, blocking the Notch signalling pathway with a γ-secretase inhibitor, DAPT, reduces DCIS mammosphere formation.[108] Taken together, these results strongly suggest that Notch pathways play a critical role in breast CSCs and, thus, may represent novel therapeutic targets to prevent recurrence of pre-invasive and invasive breast cancer. Likewise, Fan *et al.* found that the growth of neurospheres *in vitro* and the growth of tumour xenografts *in vivo* have been reduced while Notch receptors were blockaded by GSIs.[166] This study also interpreted that Notch pathway inhibition reduced proliferation and increased apoptosis resulting in the depletion of stem-like cancer cells through decreasing AKT and STAT3 phosphorylation.[166] Interestingly, there are controversial conclusions on Notch in human hematopoietic stem cells. It was reported that Jagged1-expressing osteoblasts regulated hematopoietic stem cell function through Notch1 activation.[167] Conversely, Maillard *et al.* showed that blocking Notch-mediated transcriptional activation using dominant-negative Mastermind-like1 (DNMAML), a highly specific inhibitor of canonical Notch signaling through the CSL/RBPJ-ICN-MAML complex, did not impair hematopoietic stem cell (HSC) numbers or function, suggesting that cell-autonomous canonical Notch signals are dispensable for adult HSC maintenance.[168] Similar results were observed in bone marrow progenitors and/or bone marrow stromal cells with inactivation of the Jagged1 gene, which did not impair HSC self-renewal or differentiation in all blood lineages. In addition, HSCs with Notch1-deficient were able to reconstitute mice with inactivated Jagged1 in the BM stroma, suggesting an unessential role for Jagged1-mediated Notch signaling during hematopoiesis.[169] As there are different roles of Notch in stem cells, further molecular and mechanistic studies are needed to clarify the specific roles of the Notch family in CSCs.

Notch in clinical cancer therapy

The role of Notch signaling in the pathophysiology of cancer and cancer stem cells has provided a potential therapeutic target for cancer management. Specifically, given the well-documented role of overactive Notch signaling in several solid tumors, inhibition therapy, such as using γ-secretase inhibitors or antibodies against Notches in the treatment of cancer seems to be a promising option. Phase I pharmacologic and pharmacodynamic studies of GSI MK-0752[170,171] and RO4929097[81,172] have demonstrated that toxicity was time- and dose-dependent, and rational combination would be needed to maximize clinical benefit with this agent. Common GSI-related toxicities involving diarrhea, nausea, vomiting, and fatigue are partly a result of non-selective inhibiting Notch receptors.[172–175] Studies on lung cancer therapy have demonstrated that MRK-003, a γ-secretase inhibitor, inhibited the growth and apoptosis of lung cancer cell lines, both *in vitro* and *in vivo*, through specific action on the Notch3 receptor,[176] suggesting that specific inhibition of one of the Notch receptors would be effective. In addition, Meng *et al*.[177] reported that Notch1 was up-regulated in response to oxaliplatin treatment in colon cancer. The combination of GSI with oxaliplatin significantly enhanced chemotherapeutic efficacy. Similarly, in triple negative breast cancer (TNBC), combinations of GSIs and taxanes have shown synergistic efficacy.[178] Down-regulation of Notch signaling by GSI resulted in enhanced radiosensitivity of nasopharyngeal carcinoma cells.[179] Taken together, these results suggest that combining GSIs with chemotherapy or radiotherapy may represent a novel approach. Not all Notch receptors are sensitive to GSIs, but Notch4 has been shown to be resistant to some GSIs.[180] Antibodies downregulated the expression of Notches, to positive effect in preclinical and clinical trials.[181] Based on the characteristics of the structure, function, and regulation of Notch receptors and ligands, Notch signaling as a selected target for therapy will include methods of inhibiting the expression of ligands, blocking ligand–receptor binding, and down-regulating target genes.[182,183]

Conclusion

A considerable body of evidence has implicated Notch signaling as involved in the pathogenesis and development of cancers through a variety of mechanisms. But is also raises some questions. First, incontestable evidence has indicated that Notch signaling facilitates a variety of solid tumors. It is also sobering to realize that artificial over-expression of Notch1 or Notch2 in SCLC causes a profound growth arrest,[134] highlighting that the role of Notch in cancer is dependent upon cell context and cancer type. In addition, the cross-talk between Notch signaling and other pathways appears to have the opposite effect in cancers, for example, the cross-talk between Notch and Wnt may be partly responsible for the controversial roles of Notch in cancer. Finally, four Notch receptors present distinct, even opposite, roles in tumors. Notch1 is a suppressor in embryonal brain tumors, while Notch2 contributes to the growth of tumors,[184] which may be a result of the differences in their structure, specifically the EGF-like repeats in the extracellular domain.[185] As the exact mechanism of the paradoxical roles of the Notch pathway in different cancers is still unclear, further studies are required. Interestingly, the Notch pathway has positive correlation with the maintenance and proliferation of CSCs, suggesting that targeting the Notch pathway in cancer treatment reduces the latency and recurrence of tumors, thereby decreasing the morbidity and mortality of cancer. Hence, future research should aim to decipher the complex cross-talk networks of Notch, as insight into Notch signaling will increase our ability to better design rational regimens that are more likely to be proven safe and effective.

Acknowledgments

This work was supported by the Guangxi Ministry of Education project YCSZ2012050 (HG); the National Natural Science Foundation of China (NSFC) Key Project 81130046 (JZ), NSFC81171993 (YL) and NSFC81272415 (YL); the Guangxi Key Projects 2013GXNSFEA053004 (JZ); the Guangxi Projects 1355004-5 (JZ), 2012GXNSFCB053004 (YL); the Guangxi Ministry of Education 201202ZD022 (YL), 201201ZD004 (JZ); the National Institutes of Health (NIH) Grant P01 CA093900 (ETK); and the 973 project 2010CB529405 (QZ). The authors thank Drs. Jiejun Fu and Chunlin Zou for helpful discussions and Ms. Xiaolin Zhou and Xin Huang for editing.

Disclosure

No authors report any conflict of interest.

References

1 Morgan TH. The theory of the gene. *Am Nat* 1917; **51**: 513–44.
2 Artavanis-Tsakonas S, Muskavitch MA, Yedvobnick B. Molecular cloning of Notch, a locus affecting neurogenesis in Drosophila melanogaster. *Proc Natl Acad Sci U S A* 1983; **80**: 1977–81.
3 Almén MS, Nordström KJ, Fredriksson R, Schiöth HB. Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. *BMC Biol* 2009; **7**: 50.
4 Ilagan MX, Kopan R. SnapShot: Notch signaling pathway. *Cell* 2007; **128**: 1246.
5 Weinmaster G. The ins and outs of Notch signaling. *Mol Cell Neurosci* 1997; **9**: 91–102.

6 Weng AP, Ferrando AA, Lee W *et al*. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 2004; **306**: 269–71.
7 Bolós V, Grego-Bessa J, de la Pompa JL. Notch signaling in development and cancer. *Endocr Rev* 2007; **28**: 339–63.
8 Kopan R, Ilagan MX. The canonical Notch signaling pathway: unfolding the activation mechanism. *Cell* 2009; **137**: 216–33.
9 Le Gall M, De Mattei C, Giniger E. Molecular separation of two signaling pathways for the receptor, Notch. *Dev Biol* 2008; **313**: 556–67.
10 Barolo S, Posakony JW. Three habits of highly effective signaling pathways: principles of transcriptional control by developmental cell signaling. *Genes Dev* 2002; **16**: 1167–81.
11 Gerhart J. 1998 Warkany lecture: signaling pathways in development. *Teratology* 1999; **60**: 226–39.
12 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**: 281–91.
13 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**: 8108–12.
14 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**: 207–16.
15 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**: 197–206.
16 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**: 518–22.
17 Borggrefe T, Oswald F. The Notch signaling pathway: transcriptional regulation at Notch target genes. *Cell Mol Life Sci* 2009; **66**: 1631–46.
18 Hsieh JJ, Zhou S, Chen L, Young DB, Hayward SD. CIR, a corepressor linking the DNA binding factor CBF1 to the histone deacetylase complex. *Proc Natl Acad Sci U S A* 1999; **96**: 23–8.
19 Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. Signalling downstream of activated mammalian Notch. *Nature* 1995; **377**: 355–8.
20 Kao HY, Ordentlich P, Koyano-Nakagawa N *et al*. A histone deacetylase corepressor complex regulates the Notch signal transduction pathway. *Genes Dev* 1998; **12**: 2269–77.
21 Fiúza UM, Arias AM. Cell and molecular biology of Notch. *J Endocrinol* 2007; **194**: 459–74.
22 Fortini ME, Artavanis-Tsakonas S. The suppressor of hairless protein participates in notch receptor signaling. *Cell* 1994; **79**: 273–82.
23 Wu L, Griffin JD. Modulation of Notch signaling by mastermind-like (MAML) transcriptional co-activators and their involvement in tumorigenesis. *Semin Cancer Biol* 2004; **14**: 348–56.
24 Wu L, Aster JC, Blacklow SC, Lake R, Artavanis-Tsakonas S, Griffin JD. MAML1, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. *Nat Genet* 2000; **26**: 484–9.
25 Wu L, Sun T, Kobayashi K, Gao P, Griffin JD. Identification of a family of mastermind-like transcriptional coactivators for mammalian notch receptors. *Mol Cell Biol* 2002; **22**: 7688–700.
26 Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol* 2003; **194**: 237–55.
27 Schwanbeck R, Schroeder T, Henning K *et al*. Notch signaling in embryonic and adult myelopoiesis. *Cells Tissues Organs* 2008; **188**: 91–102.
28 Zanotti S, Canalis E. Notch and the skeleton. *Mol Cell Biol* 2010; **30**: 886–96.
29 Pitsouli C, Delidakis C. The interplay between DSL proteins and ubiquitin ligases in Notch signaling. *Development* 2005; **132**: 4041–50.
30 Kageyama R, Ohtsuka T, Kobayashi T. The Hes gene family: repressors and oscillators that orchestrate embryogenesis. *Development* 2007; **134**: 1243–51.
31 Ohtsuka T, Ishibashi M, Gradwohl G, Nakanishi S, Guillemot F, Kageyama R. Hes1 and Hes5 as notch effectors in mammalian neuronal differentiation. *EMBO J* 1999; **18**: 2196–207.
32 Kunnimalaiyaan M, Vaccaro AM, Ndiaye MA, Chen H. Overexpression of the NOTCH1 intracellular domain inhibits cell proliferation and alters the neuroendocrine phenotype of medullary thyroid cancer cells. *J Biol Chem* 2006; **281**: 39819–30.
33 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**: 5925–34.
34 Weng AP, Millholland JM, Yashiro-Ohtani Y *et al*. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. *Genes Dev* 2006; **20**: 2096–109.
35 Oswald F, Liptay S, Adler G, Schmid RM. NF-kappaB2 is a putative target gene of activated Notch-1 via RBP-Jkappa. *Mol Cell Biol* 1998; **18**: 2077–88.
36 Diévart A, Beaulieu N, Jolicoeur P. Involvement of Notch1 in the development of mouse mammary tumors. *Oncogene* 1999; **18**: 5973–81.
37 Chen Y, Fischer WH, Gill GN. Regulation of the ERBB-2 promoter by RBPJkappa and NOTCH. *J Biol Chem* 1997; **272**: 14110–4.
38 Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science* 1999; **284**: 770–6.
39 Miele L. Notch signaling. *Clin Cancer Res* 2006; **12**: 1074–9.
40 Miele L, Miao H, Nickoloff BJ. NOTCH signaling as a novel cancer therapeutic target. *Curr Cancer Drug Targets* 2006; **6**: 313–23.

41 Miele L, Osborne B. Arbiter of differentiation and death: Notch signaling meets apoptosis. *J Cell Physiol* 1999; **181**: 393–409.
42 Efstratiadis A, Szabolcs M, Klinakis A. Notch, Myc and breast cancer. *Cell Cycle* 2007; **6**: 418–29.
43 Altieri DC. New wirings in the survivin networks. *Oncogene* 2008; **27**: 6276–84.
44 Ryan BM, O'Donovan N, Duffy MJ. Survivin: a new target for anti-cancer therapy. *Cancer Treat Rev* 2009; **35**: 553–62.
45 Eliasz S, Liang S, Chen Y *et al*. Notch-1 stimulates survival of lung adenocarcinoma cells during hypoxia by activating the IGF-1R pathway. *Oncogene* 2010; **29**: 2488–98.
46 Niessen K, Fu Y, Chang L, Hoodless PA, McFadden D, Karsan A. Slug is a direct Notch target required for initiation of cardiac cushion cellularization. *J Cell Biol* 2008; **182**: 315–25.
47 Heitzler P. Biodiversity and noncanonical Notch signaling. *Curr Top Dev Biol* 2010; **92**: 457–81.
48 D'Souza B, Meloty-Kapella L, Weinmaster G. Canonical and non-canonical Notch ligands. *Curr Top Dev Biol* 2010; **92**: 73–129.
49 Jin S, Mutvei AP, Chivukula IV *et al*. Non-canonical Notch signaling activates IL-6/JAK/STAT signaling in breast tumor cells and is controlled by p53 and IKKalpha/IKKbeta. *Oncogene* 2013; **32**: 4892–902.
50 Gentle ME, Rose A, Bugeon L, Dallman MJ. Noncanonical Notch signaling modulates cytokine responses of dendritic cells to inflammatory stimuli. *J Immunol* 2012; **189**: 1274–84.
51 Song JK, Giniger E. Noncanonical Notch function in motor axon guidance is mediated by Rac GTPase and the GEF1 domain of Trio. *Dev Dyn* 2011; **240**: 324–32.
52 Wu F, Stutzman A, Mo YY. Notch signaling and its role in breast cancer. *Front Biosci* 2007; **12**: 4370–83.
53 Guo S, Liu M, Gonzalez-Perez RR. Role of Notch and its oncogenic signaling crosstalk in breast cancer. *Biochim Biophys Acta* 2011; **1815**: 197–213.
54 Karamboulas C, Ailles L. Developmental signaling pathways in cancer stem cells of solid tumors. *Biochim Biophys Acta* 2013; **1830**: 2481–95.
55 Katoh M. Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. *Stem Cell Rev* 2007; **3**: 30–8.
56 Cadigan KM, Nusse R. Wnt signaling: a common theme in animal development. *Genes Dev* 1997; **11**: 3286–305.
57 Fre S, Pallavi SK, Huyghe M *et al*. Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine. *Proc Natl Acad Sci U S A* 2009; **106**: 6309–14.
58 Qi J, Zhu YQ. Targeting the most upstream site of Wnt signaling pathway provides a strategic advantage for therapy in colorectal cancer. *Curr Drug Targets* 2008; **9**: 548–57.
59 Pannequin J, Bonnans C, Delaunay N *et al*. The wnt target jagged-1 mediates the activation of notch signaling by progastrin in human colorectal cancer cells. *Cancer Res* 2009; **69**: 6065–73.
60 Sottnik JL, Hall CL, Zhang J, Keller ET. Wnt and Wnt inhibitors in bone metastasis. *Bonekey Rep* 2012; **1**: 101.
61 Galceran J, Sustmann C, Hsu SC, Folberth S, Grosschedl R. LEF1-mediated regulation of Delta-like1 links Wnt and Notch signaling in somitogenesis. *Genes Dev* 2004; **18**: 2718–23.
62 Katoh M, Katoh M. Notch ligand, JAG1, is evolutionarily conserved target of canonical WNT signaling pathway in progenitor cells. *Int J Mol Med* 2006; **17**: 681–5.
63 Estrach S, Ambler CA, Lo Celso C, Hozumi K, Watt FM. Jagged 1 is a beta-catenin target gene required for ectopic hair follicle formation in adult epidermis. *Development* 2006; **133**: 4427–38.
64 Rodilla V, Villanueva A, Obrador-Hevia A *et al*. Jagged1 is the pathological link between Wnt and Notch pathways in colorectal cancer. *Proc Natl Acad Sci U S A* 2009; **106**: 6315–20.
65 Ayyanan A, Civenni G, Ciarloni L *et al*. Increased Wnt signaling triggers oncogenic conversion of human breast epithelial cells by a Notch-dependent mechanism. *Proc Natl Acad Sci U S A* 2006; **103**: 3799–804.
66 Camps J, Pitt JJ, Emons G *et al*. Genetic amplification of the NOTCH modulator LNX2 upregulates the WNT/beta-catenin pathway in colorectal cancer. *Cancer Res* 2013; **73**: 2003–13.
67 Deregowski V, Gazzerro E, Priest L, Rydziel S, Canalis E. Notch 1 overexpression inhibits osteoblastogenesis by suppressing Wnt/beta-catenin but not bone morphogenetic protein signaling. *J Biol Chem* 2006; **281**: 6203–10.
68 Phng LK, Potente M, Leslie JD *et al*. Nrarp coordinates endothelial Notch and Wnt signaling to control vessel density in angiogenesis. *Dev Cell* 2009; **16**: 70–82.
69 Ogaki S, Shiraki N, Kume K, Kume S. Wnt and Notch signals guide embryonic stem cell differentiation into the intestinal lineages. *Stem Cells* 2013; **31**: 1086–96.
70 Kishimoto T. Interleukin-6: from basic science to medicine – 40 years in immunology. *Annu Rev Immunol* 2005; **23**: 1–21.
71 Grivennikov S, Karin M. Autocrine IL-6 signaling: a key event in tumorigenesis? *Cancer Cell* 2008; **13**: 7–9.
72 Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. *Biochem J* 1998; **334**: 297–314.
73 Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. *Eur J Cancer* 2005; **41**: 2502–12.
74 Sethi N, Dai X, Winter CG, Kang Y. Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell* 2011; **19**: 192–205.
75 Wang Y, Klijn JG, Zhang Y *et al*. Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. *Lancet* 2005; **365**: 671–9.
76 Salgado R, Junius S, Benoy I *et al*. Circulating interleukin-6 predicts survival in patients with metastatic breast cancer. *Int J Cancer* 2003; **103**: 642–6.
77 Korkaya H, Kim GI, Davis A *et al*. Activation of an IL6 inflammatory loop mediates trastuzumab resistance in

HER2+ breast cancer by expanding the cancer stem cell population. *Mol Cell* 2012; **47**: 570–84.
78 Korkaya H, Liu S, Wicha MS. Regulation of cancer stem cells by cytokine networks: attacking cancer's inflammatory roots. *Clin Cancer Res* 2011; **17**: 6125–9.
79 Sansone P, Storci G, Tavolari S *et al*. IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *J Clin Invest* 2007; **117**: 3988–4002.
80 Wongchana W, Palaga T. Direct regulation of interleukin-6 expression by Notch signaling in macrophages. *Cell Mol Immunol* 2012; **9**: 155–62.
81 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**: 292–301.
82 Mars WM, Zarnegar R, Michalopoulos GK. Activation of hepatocyte growth factor by the plasminogen activators uPA and tPA. *Am J Pathol* 1993; **143**: 949–58.
83 Ellis V, Behrendt N, Dano K. Plasminogen activation by receptor-bound urokinase. A kinetic study with both cell-associated and isolated receptor. *J Biol Chem* 1991; **266**: 12752–8.
84 Ellis V, Pyke C, Eriksen J, Solberg H, Dano K. The urokinase receptor: involvement in cell surface proteolysis and cancer invasion. *Ann N Y Acad Sci* 1992; **667**: 13–31.
85 Fisher JL, Mackie PS, Howard ML, Zhou H, Choong PF. The expression of the urokinase plasminogen activator system in metastatic murine osteosarcoma: an in vivo mouse model. *Clin Cancer Res* 2001; **7**: 1654–60.
86 Shimizu M, Cohen B, Goldvasser P, Berman H, Virtanen C, Reedijk M. Plasminogen activator uPA is a direct transcriptional target of the JAG1-Notch receptor signaling pathway in breast cancer. *Cancer Res* 2011; **71**: 277–86.
87 Huang HY, Jiang ZF, Li QX *et al*. Inhibition of human breast cancer cell invasion by siRNA against urokinase-type plasminogen activator. *Cancer Invest* 2010; **28**: 689–97.
88 Tang L, Han X. The urokinase plasminogen activator system in breast cancer invasion and metastasis. *Biomed Pharmacother* 2013; **67**: 179–82.
89 Gorantla B, Asuthkar S, Rao JS, Patel J, Gondi CS. Suppression of the uPAR-uPA system retards angiogenesis, invasion, and in vivo tumor development in pancreatic cancer cells. *Mol Cancer Res* 2011; **9**: 377–89.
90 Pappot H, Hoyer-Hansen G, Ronne E *et al*. Elevated plasma levels of urokinase plasminogen activator receptor in non-small cell lung cancer patients. *Eur J Cancer* 1997; **33**: 867–72.
91 Provost JJ, Rastedt D, Canine J *et al*. Urokinase plasminogen activator receptor induced non-small cell lung cancer invasion and metastasis requires NHE1 transporter expression and transport activity. *Cell Oncol (Dordr)* 2012; **35(2)**: 95–110.
92 Zhang J, Sud S, Mizutani K, Gyetko MR, Pienta KJ. Activation of urokinase plasminogen activator and its receptor axis is essential for macrophage infiltration in a prostate cancer mouse model. *Neoplasia* 2011; **13**: 23–30.
93 Shariat SF, Roehrborn CG, McConnell JD *et al*. Association of the circulating levels of the urokinase system of plasminogen activation with the presence of prostate cancer and invasion, progression, and metastasis. *J Clin Oncol* 2007; **25**: 349–55.
94 Duffy MJ. The urokinase plasminogen activator system: role in malignancy. *Curr Pharm Des* 2004; **10**: 39–49.
95 Choong PF, Nadesapillai AP. Urokinase plasminogen activator system: a multifunctional role in tumor progression and metastasis. *Clin Orthop Relat Res* 2003; (415 Suppl.): S46–58.
96 Mazar AP, Ahn RW, O'Halloran TV. Development of novel therapeutics targeting the urokinase plasminogen activator receptor (uPAR) and their translation toward the clinic. *Curr Pharm Des* 2011; **17**: 1970–8.
97 Nguyen DX, Bos PD, Massagué J. Metastasis: from dissemination to organ-specific colonization. *Nat Rev Cancer* 2009; **9**: 274–84.
98 Raghu H, Gondi CS, Dinh DH, Gujrati M, Rao JS. Specific knockdown of uPA/uPAR attenuates invasion in glioblastoma cells and xenografts by inhibition of cleavage and trafficking of Notch -1 receptor. *Mol Cancer* 2011; **10**: 130–145.
99 Wang Z, Li Y, Banerjee S *et al*. Down-regulation of Notch-1 and Jagged-1 inhibits prostate cancer cell growth, migration and invasion, and induces apoptosis via inactivation of Akt, mTOR, and NF-kappaB signaling pathways. *J Cell Biochem* 2010; **109**: 726–36.
100 Bin Hafeez B, Adhami VM, Asim M *et al*. Targeted knockdown of Notch1 inhibits invasion of human prostate cancer cells concomitant with inhibition of matrix metalloproteinase-9 and urokinase plasminogen activator. *Clin Cancer Res* 2009; **15**: 452–9.
101 Ellisen LW, Bird J, West DC *et al*. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* 1991; **66**: 649–61.
102 Thompson BJ, Buonamici S, Sulis ML *et al*. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. *J Exp Med* 2007; **204**: 1825–35.
103 Reschly EJ, Spaulding C, Vilimas T *et al*. Notch1 promotes survival of E2A-deficient T cell lymphomas through pre-T cell receptor-dependent and -independent mechanisms. *Blood* 2006; **107**: 4115–21.
104 Sharma VM, Calvo JA, Draheim KM *et al*. Notch1 contributes to mouse T-cell leukemia by directly inducing the expression of c-myc. *Mol Cell Biol* 2006; **26**: 8022–31.
105 Palomero T, Lim WK, Odom DT *et al*. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. *Proc Natl Acad Sci U S A* 2006; **103**: 18261–6.

106 Beverly LJ, Felsher DW, Capobianco AJ. Suppression of p53 by Notch in lymphomagenesis: implications for initiation and regression. *Cancer Res* 2005; **65**: 7159–68.
107 Bolós V, Mira E, Martínez-Poveda B *et al*. Notch activation stimulates migration of breast cancer cells and promotes tumor growth. *Breast Cancer Res* 2013; **15**: R54.
108 Farnie G, Clarke RB. Mammary stem cells and breast cancer – role of Notch signalling. *Stem Cell Rev* 2007; **3**: 169–75.
109 Speiser JJ, Ersahin C, Osipo C. The functional role of notch signaling in triple-negative breast cancer. *Vitam Horm* 2013; **93**: 277–306.
110 Stylianou S, Clarke RB, Brennan K. Aberrant activation of notch signaling in human breast cancer. *Cancer Res* 2006; **66**: 1517–25.
111 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**: 218–20.
112 Jhappan C, Gallahan D, Stahle C *et al*. Expression of an activated Notch-related int-3 transgene interferes with cell differentiation and induces neoplastic transformation in mammary and salivary glands. *Genes Dev* 1992; **6**: 345–55.
113 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**: 8530–7.
114 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**: 215–21.
115 Yabuuchi S, Pai SG, Campbell NR *et al*. Notch signaling pathway targeted therapy suppresses tumor progression and metastatic spread in pancreatic cancer. *Cancer Lett* 2013; **335**: 41–51.
116 Wang Z, Zhang Y, Li Y, Banerjee S, Liao J, Sarkar FH. Down-regulation of Notch-1 contributes to cell growth inhibition and apoptosis in pancreatic cancer cells. *Mol Cancer Ther* 2006; **5**: 483–93.
117 Hassan KA, Wang L, Korkaya H *et al*. Notch pathway activity identifies cells with cancer stem cell-like properties and correlates with worse survival in lung adenocarcinoma. *Clin Cancer Res* 2013; **19**: 1972–80.
118 Razumilava N, Gores GJ. Notch-driven carcinogenesis: the merging of hepatocellular cancer and cholangiocarcinoma into a common molecular liver cancer subtype. *J Hepatol* 2013; **58**: 1244–5.
119 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**: 3166–76.
120 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**: 4182–90.
121 Tien AC, Rajan A, Bellen HJ. A Notch updated. *J Cell Biol* 2009; **184**: 621–9.
122 Fortini ME. Notch signaling: the core pathway and its posttranslational regulation. *Dev Cell* 2009; **16**: 633–47.
123 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**: 2935–48.
124 Sethi S, Macoska J, Chen W, Sarkar FH. Molecular signature of epithelial-mesenchymal transition (EMT) in human prostate cancer bone metastasis. *Am J Transl Res* 2010; **3**: 90–9.
125 Phillips TM, Kim K, Vlashi E, McBride WH, Pajonk F. Effects of recombinant erythropoietin on breast cancer-initiating cells. *Neoplasia* 2007; **9**: 1122–9.
126 Nicolas M, Wolfer A, Raj K *et al*. Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet* 2003; **33**: 416–21.
127 Thélu J, Rossio P, Favier B. Notch signalling is linked to epidermal cell differentiation level in basal cell carcinoma, psoriasis and wound healing. *BMC Dermatol* 2002; **2**: 7.
128 Gat U, DasGupta R, Degenstein L, Fuchs E. De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. *Cell* 1998; **95**: 605–14.
129 Zhu AJ, Watt FM. Beta-catenin signalling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. *Development* 1999; **126**: 2285–98.
130 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**: 1485–95.
131 Kunnimalaiyaan M, Traeger K, Chen H. Conservation of the Notch1 signaling pathway in gastrointestinal carcinoid cells. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G636–42.
132 Kunnimalaiyaan M, Yan S, Wong F, Zhang YW, Chen H. Hairy Enhancer of Split-1 (HES-1), a Notch1 effector, inhibits the growth of carcinoid tumor cells. *Surgery* 2005; **138**: 1137–42.
133 Nakakura EK, Sriuranpong VR, Kunnimalaiyaan M *et al*. Regulation of neuroendocrine differentiation in gastrointestinal carcinoid tumor cells by notch signaling. *J Clin Endocrinol Metab* 2005; **90**: 4350–6.
134 Sriuranpong V, Borges MW, Ravi RK *et al*. Notch signaling induces cell cycle arrest in small cell lung cancer cells. *Cancer Res* 2001; **61**: 3200–5.
135 Westhoff B, Colaluca IN, D'Ario G *et al*. Alterations of the Notch pathway in lung cancer. *Proc Natl Acad Sci U S A* 2009; **106**: 22293–8.
136 Chen Y, Li D, Liu H *et al*. Notch-1 signaling facilitates survivin expression in human non-small cell lung cancer cells. *Cancer Biol Ther* 2011; **11**: 14–21.
137 Donnem T, Andersen S, Al-Shibli K, Al-Saad S, Busund LT, Bremnes RM. Prognostic impact of Notch ligands and receptors in nonsmall cell lung cancer: coexpression of Notch-1 and vascular endothelial growth factor-A predicts poor survival. *Cancer* 2010; **116**: 5676–85.

138 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**: 17761–6.
139 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**: 3427–36.
140 Nakhai H, Siveke JT, Klein B *et al*. Conditional ablation of Notch signaling in pancreatic development. *Development* 2008; **135**: 2757–65.
141 Hanlon L, Avila JL, Demarest RM *et al*. Notch1 functions as a tumor suppressor in a model of K-ras-induced pancreatic ductal adenocarcinoma. *Cancer Res* 2010; **70**: 4280–6.
142 Viatour P, Ehmer U, Saddic LA *et al*. Notch signaling inhibits hepatocellular carcinoma following inactivation of the RB pathway. *J Exp Med* 2011; **208**: 1963–76.
143 Croquelois A, Blindenbacher A, Terracciano L *et al*. Inducible inactivation of Notch1 causes nodular regenerative hyperplasia in mice. *Hepatology* 2005; **41**: 487–96.
144 van Harn T, Foijer F, van Vugt M *et al*. Loss of Rb proteins causes genomic instability in the absence of mitogenic signaling. *Genes Dev* 2010; **24**: 1377–88.
145 Qi R, An H, Yu Y *et al*. Notch1 signaling inhibits growth of human hepatocellular carcinoma through induction of cell cycle arrest and apoptosis. *Cancer Res* 2003; **63**: 8323–9.
146 Benitah SA, Valerón PF, van Aelst L, Marshall CJ, Lacal JC. Rho GTPases in human cancer: an unresolved link to upstream and downstream transcriptional regulation. *Biochim Biophys Acta* 2004; **1705**: 121–32.
147 Wilkinson S, Paterson HF, Marshall CJ. Cdc42-MRCK and Rho-ROCK signalling cooperate in myosin phosphorylation and cell invasion. *Nat Cell Biol* 2005; **7**: 255–61.
148 Sahai E, Marshall CJ. RHO-GTPases and cancer. *Nat Rev Cancer* 2002; **2**: 133–42.
149 Lefort K, Mandinova A, Ostano P *et al*. Notch1 is a p53 target gene involved in human keratinocyte tumor suppression through negative regulation of ROCK1/2 and MRCKalpha kinases. *Genes Dev* 2007; **21**: 562–77.
150 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105–11.
151 Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3**: 730–7.
152 Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res* 2005; **65**: 10946–51.
153 Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON. Isolation and functional characterization of murine prostate stem cells. *Proc Natl Acad Sci U S A* 2007; **104**: 181–6.
154 Maitland NJ, Collins AT. Prostate cancer stem cells: a new target for therapy. *J Clin Oncol* 2008; **26**: 2862–70.
155 Li C, Heidt DG, Dalerba P *et al*. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; **67**: 1030–7.
156 O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106–10.
157 Ricci-Vitiani L, Lombardi DG, Pilozzi E *et al*. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445**: 111–5.
158 Sell S, Leffert HL. Liver cancer stem cells. (Published erratum appears in *J Clin Oncol* 2008; **26**: 3819.) *J Clin Oncol* 2008; **26**: 2800–5.
159 Peacock CD, Watkins DN. Cancer stem cells and the ontogeny of lung cancer. *J Clin Oncol* 2008; **26**: 2883–9.
160 Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. (Published erratum appears in *Proc Natl Acad Sci U S A* 2003; **100**: 6890.) *Proc Natl Acad Sci U S A* 2003; **100**: 3983–8.
161 Bray SJ. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol* 2006; **7**: 678–89.
162 Joseph NM, Morrison SJ. Toward an understanding of the physiological function of Mammalian stem cells. *Dev Cell* 2005; **9**: 173–83.
163 Keith B, Simon MC. Hypoxia-inducible factors, stem cells, and cancer. *Cell* 2007; **129**: 465–72.
164 Ailles LE, Weissman IL. Cancer stem cells in solid tumors. *Curr Opin Biotechnol* 2007; **18**: 460–6.
165 Bouras T, Pal B, Vaillant F *et al*. Notch signaling regulates mammary stem cell function and luminal cell-fate commitment. *Cell Stem Cell* 2008; **3**: 429–41.
166 Fan X, Khaki L, Zhu TS *et al*. NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells* 2010; **28**: 5–16.
167 Calvi LM, Adams GB, Weibrecht KW *et al*. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 2003; **425**: 841–6.
168 Maillard I, Koch U, Dumortier A *et al*. Canonical notch signaling is dispensable for the maintenance of adult hematopoietic stem cells. *Cell Stem Cell* 2008; **2**: 356–66.
169 Mancini SJ, Mantei N, Dumortier A, Suter U, MacDonald HR, Radtke F. Jagged1-dependent Notch signaling is dispensable for hematopoietic stem cell self-renewal and differentiation. *Blood* 2005; **105**: 2340–2.
170 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**: 2307–13.
171 Fouladi M, Stewart CF, Olson J *et al*. Phase I trial of MK-0752 in children with refractory CNS malignancies: a pediatric brain tumor consortium study. *J Clin Oncol* 2011; **29**: 3529–34.
172 Tolcher AW, Messersmith WA, Mikulski SM *et al*. Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors. *J Clin Oncol* 2012; **30**: 2348–53.

173 Searfoss GH, Jordan WH, Calligaro DO *et al*. Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. *J Biol Chem* 2003; **278**: 46107–16.
174 Wong GT, Manfra D, Poulet FM *et al*. Chronic treatment with the gamma-secretase inhibitor LY-411,575 inhibits beta-amyloid peptide production and alters lymphopoiesis and intestinal cell differentiation. *J Biol Chem* 2004; **279**: 12876–82.
175 van Es JH, van Gijn ME, Riccio O *et al*. Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* 2005; **435**: 959–63.
176 Konishi J, Kawaguchi KS, Vo H *et al*. Gamma-secretase inhibitor prevents Notch3 activation and reduces proliferation in human lung cancers. *Cancer Res* 2007; **67**: 8051–7.
177 Meng RD, Shelton CC, Li YM *et al*. gamma-Secretase inhibitors abrogate oxaliplatin-induced activation of the Notch-1 signaling pathway in colon cancer cells resulting in enhanced chemosensitivity. *Cancer Res* 2009; **69**: 573–82.
178 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**: 1512–24.
179 Yu S, Zhang R, Liu F, Hu H, Wang H. Down-regulation of Notch signaling by a gamma-secretase inhibitor enhances the radiosensitivity of nasopharyngeal carcinoma cells. *Oncol Rep* 2011; **26**: 1323–8.
180 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**: 709–18.
181 Groth C, Fortini ME. Therapeutic approaches to modulating Notch signaling: current challenges and future prospects. *Semin Cell Dev Biol* 2012; **23**: 465–72.
182 Espinoza I, Miele L. Notch inhibitors for cancer treatment. *Pharmacol Ther* 2013; **139**: 95–110.
183 Shih IM, Wang TL. Notch signaling, gamma-secretase inhibitors, and cancer therapy. *Cancer Res* 2007; **67**: 1879–82.
184 Fan X, Mikolaenko I, Elhassan I *et al*. Notch1 and notch2 have opposite effects on embryonal brain tumor growth. *Cancer Res* 2004; **64**: 7787–93.
185 Yamamoto S, Charng WL, Rana NA *et al*. A mutation in EGF repeat-8 of Notch discriminates between Serrate/Jagged and Delta family ligands. *Science* 2012; **338**: 1229–32.

