Notch signalling in solid tumours: a little bit of everything but not all the time

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Abstract | The discovery of Notch in *Drosophila melanogaster* nearly a century ago opened the door to an ever-widening understanding of cellular processes that are controlled or influenced by Notch signalling. As would be expected with such a pleiotropic pathway, the deregulation of Notch signalling leads to several pathological conditions, including cancer. A role for Notch is well established in haematological malignancies, and more recent studies have provided evidence for the importance of Notch activity in solid tumours. As it is thought to act as an oncogene in some cancers but as a tumour suppressor in others, the role of Notch in solid tumours seems to be highly context dependent.

Negative selection
The intrathymic elimination of $CD4^+CD8^+$ thymocytes that express T cell receptors with high affinity for self antigens.

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A role for *NOTCH1* in human cancer was originally suggested owing to a chromosomal translocation that was found in a patient with T cell acute lymphoblastic leukaemia (T-ALL)[1]. Although this translocation is rare in patients with T-ALL, it was later discovered that most T-ALL cases harbour activating mutations in the *NOTCH1* locus[2] (BOX 1). These mutations generally result in ligand-independent proteolytic cleavage of NOTCH1 (REF. 3) and increased stability of the active Notch intracellular domain (NICD), the net result being the constitutive activation of the Notch pathway and the neoplastic transformation of T cells.

Although a causative role for Notch signalling is well established in T-ALL, a uniform model for the role of Notch signalling in tumorigenesis remains elusive. Despite the wealth of data suggesting a role for Notch in solid tumours, there is little evidence to support a causative role for Notch in the initiation of tumorigenesis in human solid cancers. Indeed, unlike in T-ALL, there is little evidence for genetic alterations in Notch genes in solid tumours. But in many solid tumours, including cancers of the breast, colon, pancreas, prostate and central nervous system, Notch signalling seems to be crucial (TABLE 1; see Supplementary information S1 (table)). Interestingly, Notch signalling also seems to have a contradictory tumour suppressor role in mouse keratinocytes, pancreatic and hepatocellular carcinoma, and small-cell lung cancer (reviewed in REF. 4). Taken together, these observations indicate that Notch is exerting its effects in solid tumours owing to the aberrant activation of the pathway. Moreover, the cellular interpretation and outcome of this aberrant Notch activity is highly dependent on contextual cues such as interactions with the tumour microenvironment and crosstalk with other signalling pathways.

What accounts for the lack of observed mutations in Notch genes in solid tumours? Insight can be derived from the T-ALL paradigm. During early T cell development, mutations in *NOTCH1* that result in constitutive activation can provide a cell survival advantage by bypassing the usual requirement for cell-to-cell engagement and so activating Notch signalling in order to evade negative selection. This provides a basis for the hypothesis that a cell in an epithelium cannot escape cell-to-cell contact, and so a wealth of opportunity exists for ligand-dependent activation of Notch signalling, making activating mutations of Notch genes less important. Therefore, in solid tumours the issue could be less one of 'constitutive' activation and more one of 'inappropriate' activation of Notch. Moreover, evidence that has been derived from studies of pancreatic cancer suggests that Notch signalling during the initial stages of tumorigenesis can prevent tumour formation, in contrast to later stages of tumour development, in which Notch activation is required[5,6]. This suggests the importance of the temporal and spatial context of Notch activity. Inappropriate activation of Notch signalling in tumorigenesis can be initiated in different ways, such as through the loss of a negative regulator or the deregulated expression of the Notch receptor and ligands, as has been reported in several solid tumours, including prostate tumours[7], pancreatic tumours[8], glioblastoma[9] and breast tumours[10].

At a glance

- A causative role for Notch signalling is well established in T cell acute lymphoblastic leukaemias (T-ALLs), which have activating mutations in the Notch genes resulting in a constitutively active pathway. By contrast, solid tumours, which have ample opportunity to activate the pathway, exhibit inappropriate activation by multiple mechanisms, such as overexpression of ligand or loss of negative regulators of the pathway.
- The role of Notch signalling in solid tumours is highly dependent on the spatial and temporal context of Notch activation, as well as the status of other signalling pathways in the cells.
- Notch signalling has opposing roles in tumorigenesis depending on the cell type. Opposite interactions of the Notch pathway have been documented with the WNT and p53 pathways. Although synergy with WNT and antagonism of the p53 pathway directs the oncogenic role of Notch, the opposite is seen in the tumour suppressor context.
- Notch signalling has a major role in the maintenance and progression of tumours by promoting epithelial to mesenchymal transition (EMT) and angiogenesis. It also confers resistance to radiation and chemotherapeutic agents.
- The knowledge of the extensive crosstalk of the Notch pathway with other pathways such as the epidermal growth factor receptor (EGFR) pathway could prove useful in developing combinatorial cancer therapies.

In the following sections, we discuss how the inappropriate activation of Notch facilitates malignant transformation and the progression of solid tumours, and how active Notch signalling can render cancer cells resistant to drug and radiation therapy.

Notch signalling

The mammalian Notch receptor family consists of four type I transmembrane receptors (termed NOTCH1–4), all of which have been implicated in human cancer. Notch proteins are synthesized as precursor forms that are cleaved by furin-like convertase (S1 cleavage) to generate the mature receptor, which is composed of two subunits. One of these subunits consists of the major portion of the extracellular domain (ECD), and the other subunit is composed of the remainder of the ECD, the transmembrane domain and the intracellular domain (ICD). These two subunits are held together by non-covalent interactions. The ECDs of Notch proteins are comprised of epidermal growth factor (EGF)-like repeats that have a role in ligand–receptor interactions. Carboxy-terminal to the EGF-like repeats are three cysteine-rich LIN12 and Notch repeats (LNRs), which prevent ligand-independent signalling, and a C-terminal hydrophobic region that mediates the interaction between the ECDs and the transmembrane domains. The NICD, which is composed of conserved protein domains, such as the ankyrin repeats and the PEST domain, is the active form of the protein and mediates Notch signalling (reviewed in REFS 11,12) (FIG. 1).

Although not completely understood, a scheme for Notch signalling has been generally accepted (reviewed in REF. 13). Notch signalling is initiated by the engagement of a Notch ligand to a Notch receptor, which is mediated by cell-to-cell contact. There are five known Notch ligands in mammals, jagged 1 (JAG1), JAG2, Delta-like 1 (DLL1), DLL3 and DLL4, which are collectively referred to as DSL proteins. Like the Notch receptors, the DSL proteins are type I transmembrane proteins. On binding to the Notch receptor, the ligand induces a conformational change, exposing the S2 cleavage site in the ECD to the metalloproteinase tumour necrosis factor-α-converting enzyme (TACE; also known as ADAM17). Following S2 cleavage, Notch undergoes a third cleavage (S3 cleavage) that is mediated by the presenilin–γ-secretase complex, which is composed of presenilin 1 (PSEN1), PSEN2, nicastrin (NCSTN), presenilin enhancer 2 (PEN2) and anterior pharynx-defective 1 (APH1). The S3 cleavage results in the release of the active NICD from the plasma membrane and its subsequent translocation into the nucleus[14]. It is the S3 cleavage that is targeted by the class of compounds known as γ-secretase inhibitors (GSIs). Therefore, treatment with GSIs blocks the terminal cleavage and release from the plasma membrane, preventing Notch signalling. Once in the nucleus, Notch concomitantly mediates the conversion of the CBF1–Su(H)–LAG1 (CSL) repressor complex into a transcriptional activation complex and the recruitment of the co-activator protein mastermind-like 1 (MAML1)[15]. Notch signalling is thought to exert its pleiotropic effects by initiating a transcriptional cascade that involves both the activation and the repression of target genes, including transcriptional regulation by epigenetic mechanisms (BOX 2). Although the details of such a transcriptional cascade are not completely realized, several well-characterized target genes have been described. Among these genes are the basic-helix–loop–helix (bHLH) transcriptional repressors hairy enhancer of split (HES) family, the hairy-related transcription factor (HRT; also known as HEY) family, Notch receptors, Notch ligands, cyclin D1 (*CCND1*) and *MYC*. Notch transcriptional activity is terminated by phosphorylation of Notch on the C-terminal PEST domain, which targets it for ubiquitylation by ubiquitin ligases, such as FBXW7 (also known as SEL10), and subsequent degradation by the proteasome (reviewed in REF. 16) (FIG. 2a). In addition, Notch signalling can be regulated by post-translational modifications on Notch or DSL proteins. Some of these factors are also deregulated in cancer (BOX 3).

Although the primary role for the DSL ligands is to initiate Notch signalling by triggering the proteolytic cascade of Notch receptors and the release of the active NICD, Notch ligands can also have distinct Notch-independent functions. Evidence suggests that DSL proteins can also undergo proteolytic cleavage, leading to the initiation of signalling events in the ligand-expressing cell[17–21] (FIG. 2b). The observation that ectopic expression of JAG1 can transform rat kidney epithelial (RKE) cells independently of Notch signalling, as well as the requirement for an intact PDZ-ligand motif in JAG1, prompted the hypothesis that the Notch–DSL pathway is in fact bidirectional[22]. In addition, it has been observed that Notch ligands undergo processing that is similar to Notch processing — and which uses the same proteolytic machinery — and results in the release of the ICD[17,18]. The jagged ICD (JICD) has been shown to activate AP1-mediated transcription, which is antagonized

Type I transmembrane receptors
Proteins that span the plasma membrane once, with the carboxy-terminal end extending into the cytoplasm.

by the NICD[17]. In many cultured cells, the ICD of the Delta-like ligand can induce growth arrest and senescence through the induction of p21 expression, and this can be overcome by the NICD. Thus, independent effects of the Delta ICD (DICD) also seem likely[23] (FIG. 2b). Although Notch-independent DSL signalling events have been reported, the physiological relevance of such signalling and its role in tumorigenesis remain to be determined.

Role of Notch in tumorigenesis

Oncogene or tumour suppressor gene? The initial evidence for the oncogenic role of Notch proteins in the transformation of epithelial cells came from mouse mammary tumour virus (MMTV)-mediated insertional mutagenesis studies[24,25]. Retroviral activation of *Int3* (now known as *Notch4*) by MMTV led to mammary tumorigenesis in infected mice. Furthermore, NOTCH4 was able to transform immortalized mammary epithelial cells in culture and drove mammary tumorigenesis in transgenic mice[25,26]. Similarly, it was shown that NOTCH1 and NOTCH2 could transform primary rodent epithelial cells in cooperation with adenoviral E1A[27]. More recent studies using models of T-ALL have demonstrated that Notch drives tumorigenesis mostly by promoting cell cycle progression and inhibiting apoptosis (reviewed in REF. 28). Consistent with our understanding of Notch signalling, these effects are thought to be the result of the transcriptional regulation of key components of the cell cycle and the tumour surveillance machinery. In contrast to these oncogenic activities, studies also suggest that Notch signalling has a tumour suppressor function in some cell types. This tumour suppressor activity is generally thought to be a result of crosstalk with other signalling pathways that govern decreased cell proliferation, increased apoptosis or the promotion of cellular differentiation. The following sections outline the various oncogenic and tumour suppressor roles of Notch in solid tumours (FIG. 3; see Supplementary information S1 (table)).

Cell cycle regulation. The first evidence that Notch signalling directly influences the cell cycle came from transformation studies on E1A immortalized RKE cells[27,29]. In these studies, Notch directly induced CCND1 expression and cyclin-dependent kinase 2 (CDK2) activity. Further studies on mammary tumorigenesis supported this work by showing that Notch promotes transformation by inducing CCND1 expression[30]. Increased levels of JAG1, which commonly occur in breast cancers, also promote cell cycle progression by inducing CCND1 through Notch signalling[31]. Interestingly, Notch overexpression failed to induce T-ALL in mice that were homozygous-null for *Ccnd3*, which is also a target of Notch[32]. Although this suggests an obligatory role for D-type cyclins in Notch-mediated transformation, *Ccnd3* probably has a broader role in tumorigenesis. MYC, a potent driver of cell cycle entry, is a direct transcriptional target of Notch and contributes to cell cycle progression in T-ALL[33,34], as well as in Notch-induced mouse mammary tumours[35]. NOTCH1 and MYC probably control two transcriptional programmes that together regulate the growth of primary T-ALL cells[35,36]. Although the major mechanism by which Notch promotes cell cycle progression is through the induction of CCND1 and MYC, the inhibition of cyclin-dependent kinase inhibitors (CDKIs) also has an important role. Notch mediates the transcriptional repression of the CDKIs p27 and p57 through HES1 in different cell types[37–39]. In T-ALL, Notch directs the transcription of the E3 ubiquitin ligase S phase kinase-associated protein 2 (SKP2), which leads to decreased p27 protein levels and increased cell proliferation[40].

Notch signalling can cooperate with other oncogenic signalling pathways. In breast epithelial cells, cooperation between Notch and RAS has been shown to exert proliferative effects and cause malignant transformation[41]; however, the exact nature of this cooperation is not clear. In astrocytic gliomas, Notch signalling has an oncogenic effect owing to crosstalk with the EGF receptor (EGFR) pathways and the subsequent activation of the PI3K–AKT pathway, KRAS, CCND1 and matrix metalloproteinase 9 (MMP9)[42]. Interaction between Notch and the JAK–signal transducer and activator of transcription (STAT) pathway also leads to a proliferative response, which may initiate tumour growth. In developmental systems such as *D. melanogaster*, crosstalk between Notch signalling and the JAK–STAT pathway is responsible for maintaining the balance between intestinal stem cell self-renewal and differentiation[43], and this mechanism may also be at work in malignant cells.

By contrast, the activation of EGFR signalling has been associated with the loss of Notch expression. Inhibition of γ-secretase can result in increased EGFR signalling and the subsequent proliferation of cells[44]. Active Notch signalling, coupled with the inhibition of multiple pathways that are mainly downstream of receptor tyrosine kinases (RTKs)[45–48], can decrease tumour cell proliferation[45–49]. In prostate cancer cells, which often have low levels of the tumour suppressor PTEN, ectopic activation of Notch inhibits proliferation concomitantly with an increase in the levels of PTEN, suggesting that PTEN is under the control of Notch[50,51]. However, it is not yet known how Notch regulates the expression of PTEN to inhibit tumour formation while also inducing epithelial to mesenchymal transition (EMT) and cellular invasion[52]. In human and mouse epithelial cell

Box 1 | **Genetic alterations that affect the activity of Notch**

The first genetic alteration that identified a role for Notch in T cell acute lymphoblastic leukaemia (T-ALL) was the chromosomal translocation t(7;9)(q34;q34.3), which results in the constitutive expression of the intracellular domain of NOTCH1, leading to cell proliferation and the formation of lymphoma[1]. Another translocation affecting Notch signalling is t(11;19)(q21;p13), which results in the formation of a fusion gene between mucoepidermoid carcinoma translocated 1 (*MECT1*) and mastermind-like 2 (*MAML2*), which are located at chromosomes 19p13 and 11q21, respectively. The MECT1–MAML2 fusion protein can activate Notch target genes independently of ligand stimulation[150] and can also activate cyclic AMP (cAMP)-responsive genes independently of any external stimulus[151]. This chromosomal abnormality is seen in mucoepidermoid cancer in the salivary gland[150], bronchopulmonary mucoepidermoid carcinoma[151], cervical mucoepidermoid carcinoma[152] and clear cell hidradenoma of the skin[153].

Table 1 | **Multiple roles of Notch signalling in solid tumours***

Tumour type	Oncogenic	Tumour suppressor	Tumour progression	Tumour maintenance	Drug resistance
Breast	✓	✓	✓	✓	✓
Colorectal	✓		✓		✓
Prostate		✓	✓		
Liver		✓	✓		✓
Pancreatic	✓		✓		✓
Glioblastoma		✓	✓	✓	✓
Cervical	✓		✓		✓
Oral SCC	✓	✓			
Skin		✓			
Head and neck					✓
Medulloblastoma			✓	✓	
Melanoma			✓	✓	
Lung	✓	✓	✓		

SCC, squamous cell carcinoma. *An expanded version of this table with descriptions and a full reference list is provided as Supplementary information S1 (table) (see Further information). Ticks indicate that a role for Notch has been observed in the corresponding tumour, whereas blank cells indicate that a role for Notch has not been observed in the tumour.

lines, Notch activity, together with transforming growth factor-β (TGFβ) signalling, can cause cell cycle arrest. TGFβ signalling leads to an induction in the expression levels of p21 and JAG1. The increased levels of JAG1 activate Notch signalling, which sustains the levels of p21, resulting in cell cycle arrest[53] (FIG. 3). However, the opposite relationship between Notch and TGFβ signalling has been observed in breast and cervical cancer cells. Breast cancer cells that express the NOTCH4 ICD are resistant to TGFβ-mediated growth arrest, but treating these cells with GSIs can resensitize them[54]. In cervical cancer cells, NOTCH1 signalling confers resistance to the growth inhibitory effects of TGFβ[55]. These opposing actions of Notch and TGFβ crosstalk seem to be both cell type specific and Notch paralogue dependent.

It is likely that a complex combination of factors determines the pro-tumorigenic or antitumorigenic effects of Notch crosstalk, including multiple interactions with the tumour microenvironment. For example, Notch signalling has a tumour suppressor effect in skin epithelial cells. Loss of *Notch1* in epidermal keratinocytes impairs skin barrier integrity and creates a wound-like niche that promotes tumorigenesis in a non-cell autonomous manner. Using a chimeric mouse model, it was demonstrated that in such a tumour-promoting microenvironment, expression of NOTCH1 in keratinocytes was insufficient to suppress this tumour-promoting effect, emphasizing the importance of crosstalk between this barrier-defective epidermis and its stroma[56]. It has also been demonstrated that loss of Notch signalling in the skin leads to improper epidermal differentiation and a defective skin barrier, resulting in inflammation and lymphoproliferative and myeloproliferative disorders[57,58]. This emphasizes that Notch signalling in the microenvironment can have a tumour suppressive effect.

Inhibition of apoptosis. Inhibition of apoptosis is an essential step in tumorigenesis. One of the key mechanisms by which Notch inhibits apoptosis is through the negative regulation of p53 and PTEN. Contrary to the positive regulation of PTEN by Notch in prostate cancer cells, the inhibition of Notch by GSIs in T-ALL cells increases PTEN expression. This is probably due to the decreased expression of HES1, which is a negative regulator of PTEN[59]. Decreased PTEN activity results in the activation of PI3K–AKT signalling through mTOR, which leads to the phosphorylation of MDM2 and culminates in the inhibition of p53 (REF. 60). In breast epithelial cells, the expression of active Notch results in the activation of the PI3K–AKT pathway by an autocrine loop, and so prevents apoptosis[61]. However, the activation of PI3K–AKT pathway is not accompanied by the downregulation of PTEN, suggesting that the repression of PTEN by Notch (via HES1) is highly context dependent[61,62]. Ectopic expression of NOTCH1 can also inhibit p53 activity by blocking its nuclear translocation or by preventing the serine phosphorylation that is necessary for p53 activation[63]. In T-ALL, Notch seems to disrupt the ARF–MDM2–p53 tumour surveillance pathway through the repression of ARF expression[64], which results in decreased apoptosis. A similar mechanism in solid tumours has not yet been described.

By contrast, evidence suggests that Notch signalling can induce apoptosis by increasing p53 activity in some cell types (reviewed in REF. 49). In human keratinocyte tumours, studies have shown that *NOTCH1* expression is under the direct transcriptional control of p53 (REF. 45). In hepatocellular carcinoma, ectopic expression of NOTCH1 increases the sensitivity of cancer cells to p53-mediated apoptosis by reducing proteasomal degradation of p53 by the AKT–MDM2 pathway. This in turn induces the expression of death receptor 5

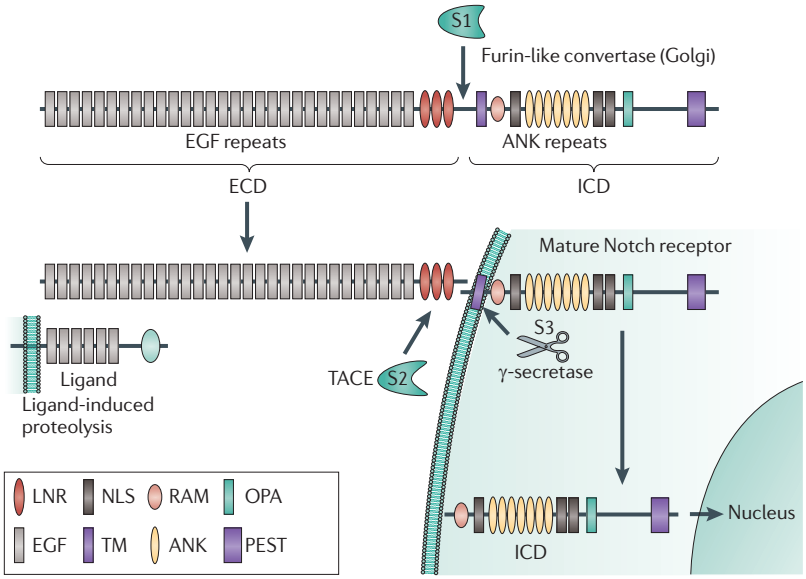

Figure 1 | **Structural organization and proteolytic processing of the Notch receptor.** Notch proteins are synthesized as precursor forms that are cleaved by furin-like convertase (S1 cleavage) to generate the mature receptor, which is composed of two subunits that are held together by non-covalent interactions. The extracellular domain (ECD) of the Notch protein is comprised of epidermal growth factor (EGF)-like repeats, three cysteine-rich LIN12 and Notch repeats (LNRs), followed by a carboxy-terminal hydrophobic region. The Notch intracellular domain (NICD) is composed of conserved protein domains: namely, the RBP-Jκ-associated module (RAM) domain, ankyrin (ANK) repeats, nuclear localization signals (NLSs) and the PEST domain. The general domain organization of the Notch proteins, with the details of NOTCH1, is shown. However, there are differences observed among the four receptors (reviewed in REF. 14). On binding to the Notch receptor, the ligand induces a conformational change, exposing the S2 cleavage site in the ECD to the metalloproteinase tumour necrosis factor-α-converting enzyme (TACE; also known as ADAM17). Following S2 cleavage, Notch undergoes a third cleavage (S3) that is mediated by the presenilin–γ-secretase complex, which is composed of presenilin 1 (PSEN1), PSEN2, nicastrin (NCSTN), presenilin enhancer 2 (PEN2) and anterior pharynx-defective 1 (APH1). The S3 cleavage results in the release of the active NICD from the plasma membrane and the subsequent translocation into the nucleus. ICD, intracellular domain; OPA, polyglutamine repeat-containing region; TM, transmembrane.

(DR5; also known as TNFRSF10B), resulting in cell death and the inhibition of tumour formation[46]. It is possible that p53 is activated merely as a cellular response to Notch-induced proliferation, which is analogous to the effect of other oncogenes such as mutant RAS or E1A.

There are also examples from studies on cervical cancer and Ewing's sarcoma in which Notch activates p53 (reviewed in REF. 49). In some human papilloma virus (HPV)-positive cervical cancer cell lines (such as HeLa), ectopic expression of the NICD results in the downregulation of HPV E6 and E7 transcription by decreasing AP1 activity, leading to the activation of p53, the inhibition of RB hyperphosphorylation and growth arrest[47,48]. Conversely, Notch inhibits apoptosis in cervical cancer cells through the activation of nuclear factor-κB (NF-κB)[65,66]. Studies in human and mouse T-ALL, and in other cell types, have shown that Notch induces the transcription of NF-κB pathway components, which may operate as a feedforward activation of NF-κB activity. A physical interaction between the NICD and the inhibitor of NF-κB kinase (IKK) complex has also been described, resulting in the activation of NF-κB (reviewed in REF. 67).

Reprogramming of differentiation. A balance between the proliferation of undifferentiated cells and their differentiation into mature cell types is key to maintaining tissue homeostasis. Under normal conditions, the programmes that govern differentiation and proliferation are tightly regulated by many 'cues' in the cellular milieu. Signalling pathways, such as those triggered by growth factors, Notch, WNT and Hedgehog (HH), act together to coordinately regulate these events. Inappropriate activation of any of these pathways can result in deregulated proliferation and differentiation programmes that lead to tumorigenesis. Crosstalk between Notch signalling and WNT signalling has been shown to initiate tumorigenesis mainly by disrupting the balance between progenitor cell proliferation and differentiation, thus maintaining cells in an undifferentiated state[68]. The WNT pathway can be activated in a number of ways, including through the constitutive activation of β-catenin owing to mutations in adenomatous polyposis coli (APC) or AXIN[69–72]; the silencing of genes that express inhibitory WNT ligands[73,74]; the overexpression of WNT receptor or ligands[75–78]; and the activating mutations in low-density lipoprotein receptor-related protein 5 (LRP5)[79]. For example, *Apc*-mutant mice develop multiple intestinal tumours owing to the constitutive activation of β-catenin. Blocking Notch signalling in these mice by GSI treatment results in the differentiation of the proliferative cells into more differentiated goblet cells, suggesting that Notch signalling might have a role in inhibiting differentiation and therefore may play a part in β-catenin-driven tumorigenesis[80]. Several lines of evidence suggest that Notch and WNT interact genetically, and there are direct physical associations between components of each pathway[81–84]. For example, β-catenin has been shown to directly bind the NICD, resulting in an increased transcriptional output of target genes[84]. In addition, MAML1 has been reported to function as a co-activator for β-catenin-dependent transcription[85], raising the possibility that signalling pathways can converge through common components.

In the skin, however, Notch suppresses tumorigenesis by blocking WNT signalling, thereby driving cells towards a more differentiated phenotype. In keratinocytes, WNT–β-catenin signalling has been associated with malignancies and with the maintenance of multipotent stem cell populations, so it is possible that the inhibition of the WNT pathway is sufficient to drive these cells towards a more differentiated phenotype. NOTCH1 activation in keratinocytes results in the repression of β-catenin signalling. Deletion of *Notch1* in the mouse epidermis results in inappropriate activation of β-catenin, and the formation of skin tumours[86]. Notch can also downregulate the expression of the WNT ligands *Wnt3* and *Wnt4* through HES1 and p21 (REF. 87), providing further mechanisms through which Notch can suppress tumorigenesis by inhibiting the WNT pathway. Although assiduously investigated, the mechanism of crosstalk between these two pathways and their interactions in tumorigenesis remain unclear (FIG. 3).

Other pathways may also crosstalk with Notch to block differentiation and to drive tumorigenesis. In pancreatic adenocarcinoma, interaction between Notch

Exocrine pancreas
The portion of the pancreas that secretes digestive enzymes that are then passed on to the small intestine.

and RAS–MAPK signalling has been implicated in the initiation of tumours. *NOTCH1* is induced by KRAS signalling, and this results in dedifferentiation or in the inhibition of differentiation in the exocrine pancreas, leading to the formation of pancreatic intraepithelial neoplasia (PanIN)[88,89]. These lesions accumulate further genetic alterations and form aggressive pancreatic ductal adenocarcinoma (PDAC)[88,89]. Interestingly, it has been hypothesized that under physiological conditions Notch can act as a negative regulator of RAS signalling and can induce the differentiation of several pancreatic cell types[90], thereby creating a context in which Notch functions as a tumour suppressor. This is supported by a recent study that demonstrated that Notch can function as a tumour suppressor in pancreatic cancers, in which deleting *Notch1* in the context of activated KRAS resulted in enhanced tumour formation in mouse models[5]. These studies underscore the hypothesis that the outcome of Notch signalling in tumorigenesis mostly depends on the temporal and spatial context in a given tissue.

Notch in tumour progression

As well as influencing tumour initiation, Notch is also important for aspects of tumour progression, including angiogenesis, EMT-driven metastatic growth and the maintenance of cancer stem cells.

Regulation of angiogenesis. Notch receptors and ligands are widely expressed in the vasculature, suggesting the importance of the Notch signalling pathway in angiogenesis. During normal angiogenesis, vascular endothelial growth factor (VEGF) drives the budding of new vessels by increasing the number of DLL4-expressing tip cells that bud out of a pre-existing vessel[91]. Although these endothelial cells are non-proliferative, they are followed by several motile, proliferative endothelial tube cells, which express Notch and form the lumen of the new vessel. DLL4 on the tip cells signals through Notch on the adjacent tube cells to decrease VEGF-induced sprouting and branching by downregulating VEGF receptor 2 (VEGFR2)[92,93]. In this manner, DLL4 inhibits angiogenesis by a negative feedback loop with VEGF (FIG. 4).

In the hypoxic tumour environment, tumour cells secrete large amounts of VEGF, which results in the expression of comparatively higher levels of DLL4 by endothelial cells in the stroma[94,95]. Subsequently blocking VEGF activity in such tumours resulted in decreased DLL4 expression in tumour endothelial cells[96,97]. The close relationship between VEGF and DLL4 expression led to the examination of the effect of blocking DLL4-mediated Notch signalling on adjacent endothelial cells, which resulted in a substantial reduction in tumour growth. Surprisingly, this was associated with an increase in vessel formation[98], possibly because DLL4 is the factor responsible for the downregulation of VEGF-induced angiogenesis. This vasculature was non-functional, suggesting that DLL4–Notch is responsible for some specialized functions in the vessels that form in response to VEGF, such as the development of the vessel lumen[98]. These results suggest that in the future it could be useful to combine VEGF inhibitors and Notch signalling inhibitors in anti-angiogenic therapy (reviewed in REF. 96).

DLL4 and JAG1 have distinct roles during angiogenesis, and they maintain a balance between endothelial cell sprouting and the formation of new vessels. Spatiotemporal regulation of Notch activation during this process is brought about by Fringe proteins[99]. This family of *N*-acetylglucosaminidyl transferases (comprised of lunatic fringe (LFNG), radical fringe (RFNG) and manic fringe (MFNG)) modulates the activity of Notch proteins through the glycosylation of the EGF-like repeats. Studies from *D. melanogaster* indicate that the Fringe proteins inhibit Serrate (*D. melanogaster* jagged homologue)-dependent Notch activation and potentiate Delta-dependent Notch activation[100] (FIG. 2a). This mechanism might also operate in other Notch-controlled biological processes, such as cancer progression and tumour angiogenesis.

Endothelial cell migration is an essential step in the production of new blood vessels. Studies in developmental systems demonstrate that the TGFβ and bone morphogenetic protein (BMP) pathways interact with the Notch pathway through SMADs, leading to alterations in endothelial cell migration. Although there is little evidence for an interaction between Notch and TGFβ in tumour angiogenesis, studies in developmental model systems suggest that a mechanism through which Notch may promote tumour growth is the repression of TGFβ-induced inhibition of endothelial cell growth[101]. In addition, some BMP family members can induce the expression of *Hey1* (also known as *Herp2*) synergistically with Notch. HEY1 then negatively regulates the activity of ID1,

Box 2 | Notch and epigenetic regulation in *Drosophila melanogaster*

Epigenetic regulation of cancer has gained considerable importance over the past few years. The reversibility of these changes, unlike genetic alterations, makes them promising targets for therapy. Epigenetic silencing of the *Notch* locus by histone methylation from Polycomb group (PcG) proteins is a mechanism through which the activity of Notch is kept under check in the *Drosophila melanogaster* eye[154]. Although evidence is very limited, there are also indications of epigenetic silencing that is mediated by Notch at its target loci. Using the *D. melanogaster* eye as a model system, Ferres-Marco *et al.*[155] showed that Notch activation cooperates with the overexpression of the Polycomb epigenetic silencers Pipsqueak and Lola in tumorigenesis. Collectively, these events result in the silencing of genes such as Retinoblastoma-family protein (*Rbf*), resulting in the formation of metastatic tumours. This began to unravel the crosstalk between the Notch pathway and the epigenetic pathways in growth control and tumorigenesis. However, whether Notch activation can directly modulate the expression and activity of these epigenetic silencers is yet to be established.

Providing an additional link between Notch signalling and epigenetic regulation, the repression of Notch target genes during *D. melanogaster* development is caused by modulating the chromatin structure, probably through histone chaperones. ASF1 is a histone chaperone that has been found to bind and inactivate Notch target loci by interacting with Su(H)/H (the *D. melanogaster* homologue of the mammalian CSL complex) and removing H3K4me3 (REF. 156). It is unclear whether ASF1 can target all loci that contain binding sites for Su(H)/H[157] or whether it may be a target of Notch signalling, thus resulting in negative feedback regulation. Identification of a similar mechanism in cancer would greatly aid the development of a strategy to disrupt Notch activity at the transcriptional level.

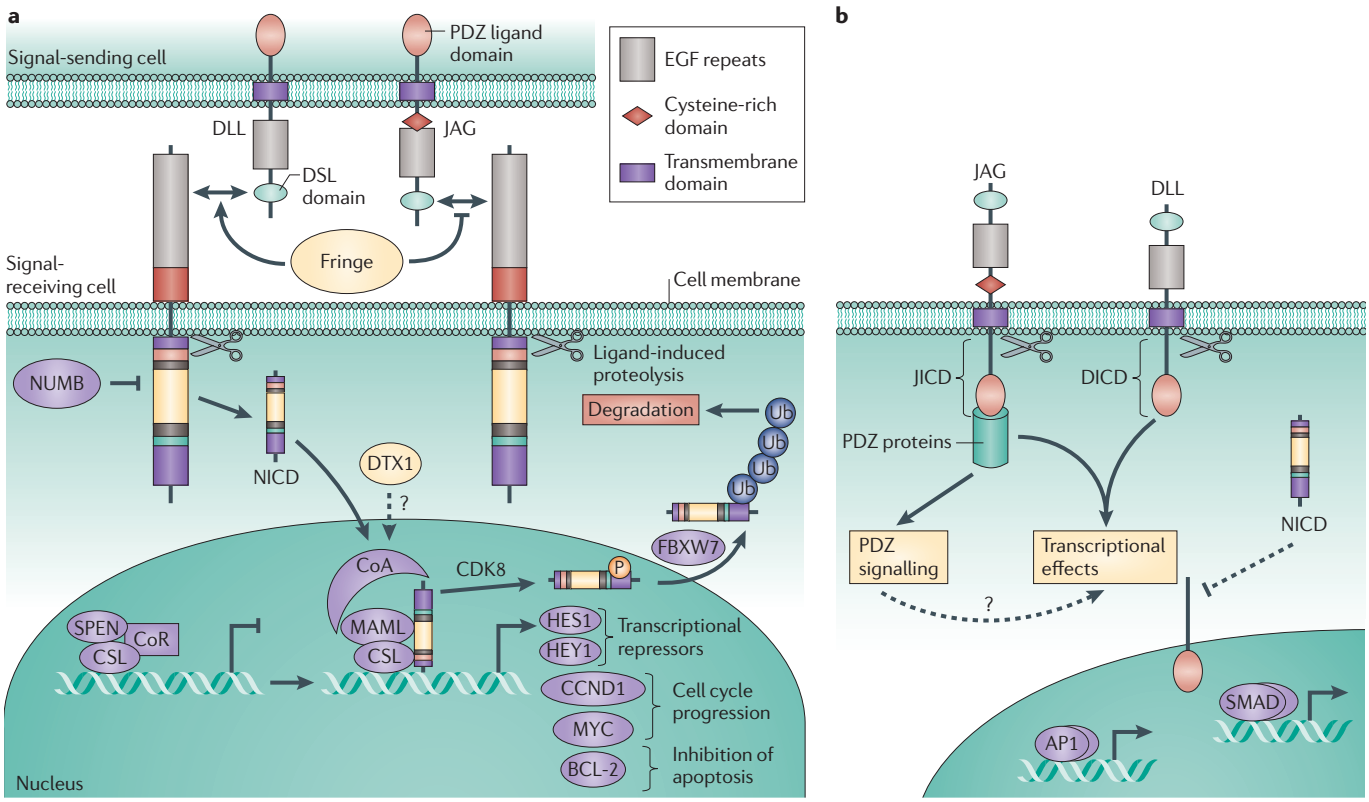

Figure 2 | **Signal transduction from Notch receptors and ligands. a** | Signal transduction from Notch receptors is shown. Notch signalling is activated by interaction between the ligand-expressing cell and the Notch-expressing cell, followed by proteolytic cleavage that releases the Notch intracellular domain (NICD) (FIG. 1). Before activation of Notch signalling, CBF1–Su(H)–LAG1 (CSL) is bound to DNA along with co-repressors (CoRs) such as MSX2-interacting protein (SPEN; also known as MINT and SHARP). On activation of Notch, the NICD recruits the co-activator (CoA), mastermind-like 1 (MAML1) and others, and thus converts the CSL-repressor complex into a transcriptional activator complex and drives the transcription of target genes. The signal is terminated by phosphorylation (P) of the PEST domain of the NICD, followed by ubiquitylation (Ub) by FBXW7 (also known as SEL10) and proteasomal degradation. Note that when the extracellular domain of Notch is glycosylated by Fringe proteins, the binding between Notch and Delta-like (DLL) is favoured and jagged (JAG) can no longer bind to and activate Notch. Deltex 1 (DTX1) inhibits Notch activity by preventing the recruitment of CoAs. It could also mediate CSL-independent effects of Notch. NUMB promotes ubiquitylation of the membrane-bound NOTCH1 and targets the NICD for proteasomal degradation. **b** | Signal transduction from Notch ligands is shown. Proteolytic cleavage releases the intracellular domain (ICD) of the Notch ligands. The PDZ ligand (PDZL) domain interacts with PDZ proteins, resulting in a signalling cascade. The ICD can also enter the nucleus and regulate transcription, possibly through interactions with AP1 or the SMAD proteins. This transcriptional regulation may be antagonized by the NICD. Dashed arrows indicate poorly understood mechanisms. CCND1, cyclin D1; CDK8, cyclin-dependent kinase 8; DICD, Delta ICD; EGF, epidermal growth factor; HES1, hairy enhancer of split 1; JICD, jagged ICD.

a promoter of endothelial cell migration[102]. This results in the inhibition of endothelial cell migration and functions as a crucial switch downstream of the Notch and BMP pathways[102].

EMT. The growth of solid tumours is highly dependent on their interaction with the microenvironment, which provides a favourable milieu for their growth and progression. These tumour–microenvironment interactions have an important role in regulating EMT (FIG. 4). The phenomenon of EMT occurs when epithelial cells undergo several morphological changes and take on a mesenchymal phenotype, including decreased adhesion, increased production of extracellular matrix components, increased migration, increased resistance to apoptosis and invasiveness. EMT is a prerequisite for the tumour cells to cross the basement membrane, enter into circulation and result in distant metastases (reviewed in REF. 103) (FIG. 4).

Recent studies have suggested that Notch can drive EMT by upregulating the expression of two target genes, *SNAIL* (also known as *SNAI1*) and *SLUG* (also known as *SNAI2*), which are transcriptional repressors of *CDH1*, the gene encoding E-cadherin. In breast cancer, JAG1 activation of Notch signalling induces EMT through the upregulation of *SLUG*[104]. A study of 154 prostate tumour samples showed an association between high expression of JAG1 and increases in metastases and tumour recurrence[7]. This study also suggested that the pro-metastatic activity of JAG1 is mediated by the induction of EMT through the AKT signalling pathway[7]. Notch might also synergize with hypoxia-inducible

Box 3 | **Notch regulators and tumorigenesis**

Several processes, including proteolysis, glycosylation, ubiquitylation and phosphorylation, control Notch activation. Aberrant activation of the Notch pathway can be caused by the overexpression of ligands or factors that activate the receptor or by the loss of negative regulators. Some of these are deregulated in cancers, resulting in aberrant Notch signalling.

Although ubiquitylation of proteins is generally associated with degradation, it also has a role in signal transduction by facilitating receptor activation and endocytosis, as is seen in ligand-dependent Notch signalling. For example, the ubiquitin ligase skeletrophin (also known as MIB2), which ubiquitylates jagged 2 (JAG2), is overexpressed in multiple myeloma, facilitating the cleavage of NOTCH1 and activating Notch-mediated transcription in stromal cells[158]. In melanoma, however, the expression of skeletrophin is lost, through loss of heterozygosity (LOH), promoter methylation or downregulation by SNAIL (also known as SNAI1), thus contributing to tumour suppression[159].

FBXW7 (also known as SEL10) is the substrate-recognition subunit of an E3 ubiquitin ligase complex that degrades Notch proteins[160] (FIG. 2a). FBXW7 is thought to be a tumour suppressor because it is deregulated, lost or mutated in several cancers, including colorectal cancer, cholangiocarcinomas and endometrial cancers[161,162]. Mutations at hot spots such as Arg465 and Arg479 result in the abrogation of substrate recognition and the inappropriate stabilization of several oncoproteins, including Notch[161]. FBXW7 function is also compromised by the latency-associated nuclear antigen (LANA) of the Kaposi's sarcoma virus, which binds to the carboxyl terminus of FBXW7, preventing its association with the Notch intracellular domain (NICD). As a result, NICD is stabilized and has increased activity, leading to the proliferation of the virus-infected cells[163].

NUMB and NUMB-like proteins function as signalling inhibitors for Notch by targeting the membrane-bound Notch for degradation following activation[164]. Loss of NUMB has been associated with breast carcinogenesis, and possibly results in the stabilization and hyperactivation of Notch[165]. In addition, NUMB binds to p53 and MDM2 to prevent ubiquitylation of p53. Thus, loss of NUMB in a large proportion of breast cancers can result in increased Notch activity and loss of p53 and an aggressive tumour phenotype with poor prognosis[166].

Another important, but not well understood, regulator of Notch signalling is Deltex. This was originally identified as a positive regulator of Notch signalling in *Drosophila melanogaster*[167]. The human homologue DTX1 (also known as deltex 1) was subsequently identified[168]. Although Deltex has been demonstrated to inhibit Notch activity by preventing the recruitment of co-activators to the CBF1–Su(H)–LAG1 (CSL)–Notch–MAML complex[169], it could function as a positive regulator of Notch signalling independently of CSL in some cell types[170] (FIG. 2a).

factor 1α (HIF1A) and HIF2A to induce EMT and therefore increase metastasis. Blocking either HIF or the Notch co-activator MAML1 in breast, colon or cervical cancer cells reduced the invasion and metastatic ability of these cells[105,106]. Furthermore, crosstalk between Notch and TGFβ is important for the initiation of EMT, as Notch signalling is required to sustain TGFβ-induced HEY1 expression[107].

Although research suggests that EMT is a prerequisite for metastases, recent evidence indicates that EMT that is mediated by Notch or any other factors can give rise to a stem cell-like phenotype, including increased resistance to apoptosis and anoikis[108].

Cancer stem cells. Cancer stem cells (CSCs; also known as tumour-initiating cells) were first described as a multipotent subpopulation of acute myeloid leukaemia cells[109] that can self-renew symmetrically or that can divide asymmetrically to produce daughter cells that continue to proliferate and so sustain tumour growth[110,111]. Recent studies have also identified CSCs in many solid tumours[112–120]. These cells have mostly been isolated on the basis of the expression of various cell surface markers, the relevance of which remains controversial. CSCs have been proposed to be resistant to radiation and chemotherapy, possibly owing to their elevated DNA damage response, their low proliferation rate[121] or their increased expression of ABC transporters[121–123].

Notch regulates the self-renewal properties and differentiation states of various cell types, including stem cells. Interaction between HIF1A and Notch has been shown to have a role in maintaining neuronal precursors in an undifferentiated state, and aberrant functioning of these cells can result in the formation of medulloblastomas[124]. Inhibition of Notch signalling or HIF1A in these cells results in their differentiation, suggesting a role for HIF1A-induced Notch signalling in maintaining stem cell characteristics[124,125]. Aberrant activation of Notch signalling by a DSL peptide has been shown to increase the self-renewal capacity of normal mammary stem cells, leading to a tenfold increase in mammosphere formation[126]. Breast CSC populations show an upregulation of Notch gene expression, and blocking Notch activity using a GSI or a neutralizing antibody to NOTCH4 reduced the mammosphere-forming ability of these cells in culture[127,128]. Likewise, brain tumour stem cells have also been shown to overexpress NOTCH1, and overexpression of NOTCH1 in human glioma cell lines increased the formation of neurospheres[129]. It is thought that Notch signalling in these neurospheres enhances their self-renewal capacity while inhibiting their differentiation into glial and neural progenitor cells[130–132]. Blocking the Notch signalling pathway with a GSI decreased the growth of neurospheres *in vitro* and the growth of tumour xenografts *in vivo*. This study also suggested that blocking Notch activity results in the decreased phosphorylation of AKT and STAT3, leading to decreased CSC proliferation and increased apoptosis[133].

A considerable body of evidence has implicated Notch signalling in many processes that are linked to the progression and maintenance of the tumour

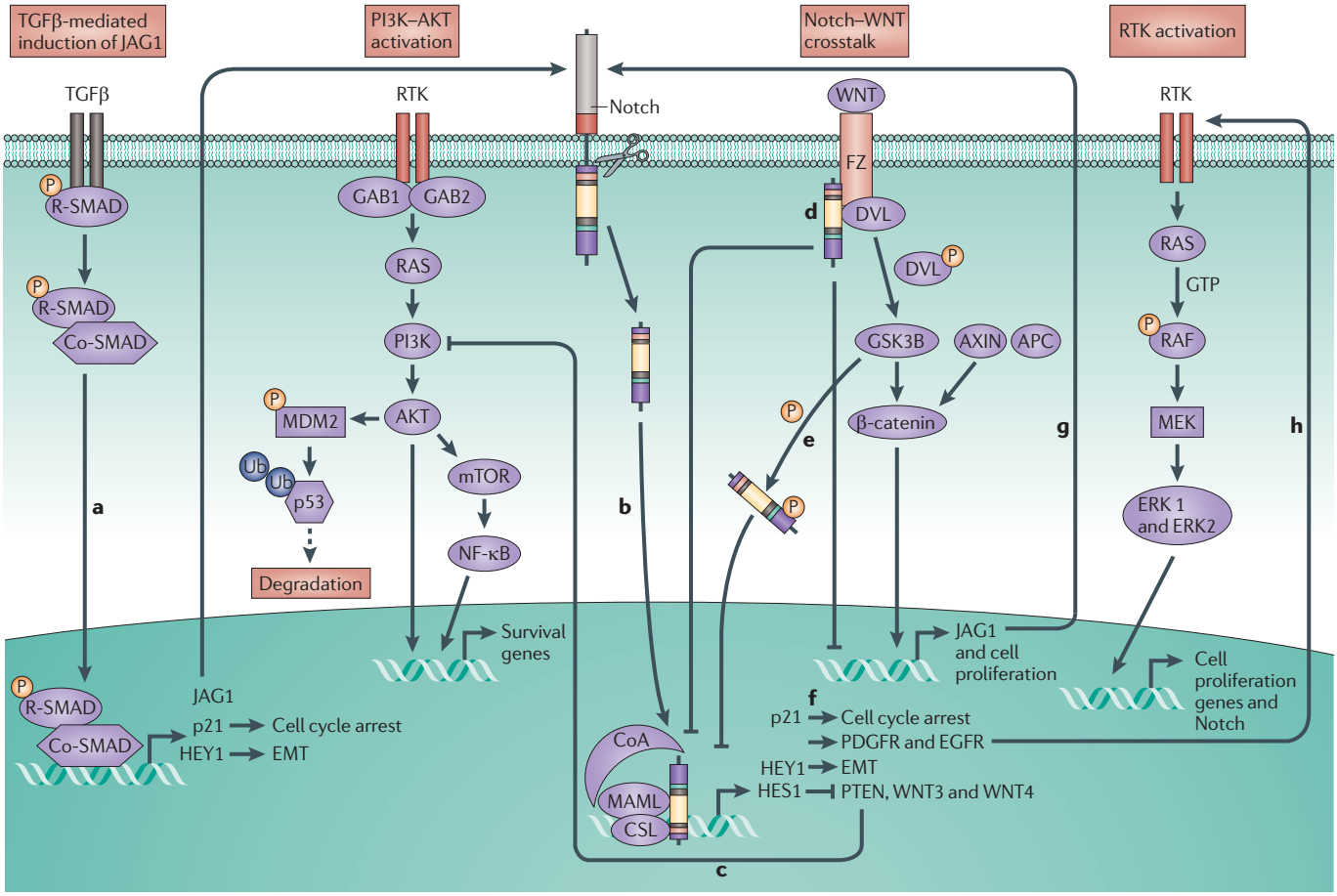

Figure 3 | **Oncogenic and tumour suppressive interactions of Notch.** Cleavage of the Notch intracellular domain (NICD) initiates a signalling cascade that interacts with other oncogenic and tumour suppressive pathways at multiple points. Jagged 1 (JAG1) is transcriptionally induced by the transforming growth factor-β (TGFβ) pathway (part **a**), which in turn activates Notch in an adjacent cell. Both TGFβ and Notch signalling lead to the induction of the cyclin-dependent kinase inhibitor p21, resulting in cell cycle arrest. HEY1 is another target of both pathways and is a mediator of the epithelial to mesenchymal transition (EMT). Notch signalling downregulates the expression of PTEN via the induction of hairy enhancer of split 1 (HES1) (part **b**), leading to the activation of the pro-survival PI3K–AKT pathway (part **c**). Binding of the NICD to a dishevelled protein (DVL) inhibits both the Notch and WNT pathways (part **d**). Phosphorylation (P) of Notch by glycogen synthase kinase 3β (GSK3B) inhibits Notch-mediated transcription (part **e**). Notch signalling inhibits the WNT ligands through the induction of HES1, thereby inhibiting the tumorigenic effects of WNT signalling (part **f**). By contrast, JAG1 is a transcriptional target of WNT, leading to WNT-mediated activation of Notch signalling (part **g**). Notch activates receptor tyrosine kinase (RTK) pathways by inducing the expression of the RTKs epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor (PDGFR) (part **h**), leading to activation of cell proliferation genes, as well as positive feedback to Notch signalling. The interactions depicted in this figure are from a variety of systems. The specific interactions among the pathways are highly context dependent. APC, adenomatous polyposis coli; CoA, co-activator; Co-SMAD, common mediator SMAD; CSL, CBF1–Su(H)–LAG1; FZ, frizzled; GAB, GRB2-associated-binding protein; MAML, mastermind-like; NF-κB, nuclear factor-κB; R-SMAD, receptor-regulated SMAD; Ub, ubiquitylation. Dashed arrow indicates a poorly understood mechanism.

phenotype. Clearly, in several distinct tumour types, abrogation of Notch signalling affects these processes and tumour growth. However, what remains unresolved is the relationship between these processes as mediated by Notch in any given tumour. For example, is the control of EMT by Notch in breast cancer linked to its role in promoting self-renewal of the CSCs and metastases? In other words, does Notch signalling alone direct these cell processes in a tumour or is the outcome of Notch signalling dependent on other crosstalk signals (FIG. 4)?

Notch and drug resistance

A major survival advantage that cancer cells can acquire is resistance to chemotherapeutic agents. This occurs mainly by activating survival pathways or by inhibiting apoptotic pathways, and Notch signalling is a major regulator of these survival pathways, through mechanisms that may be similar to its role in tumorigenesis (FIG. 3). For example, treatment of colorectal cancer with oxaliplatin activates the Notch pathway and pro-survival pathways, such as PI3K–AKT. Moreover, blocking Notch activation using GSIs sensitizes cells

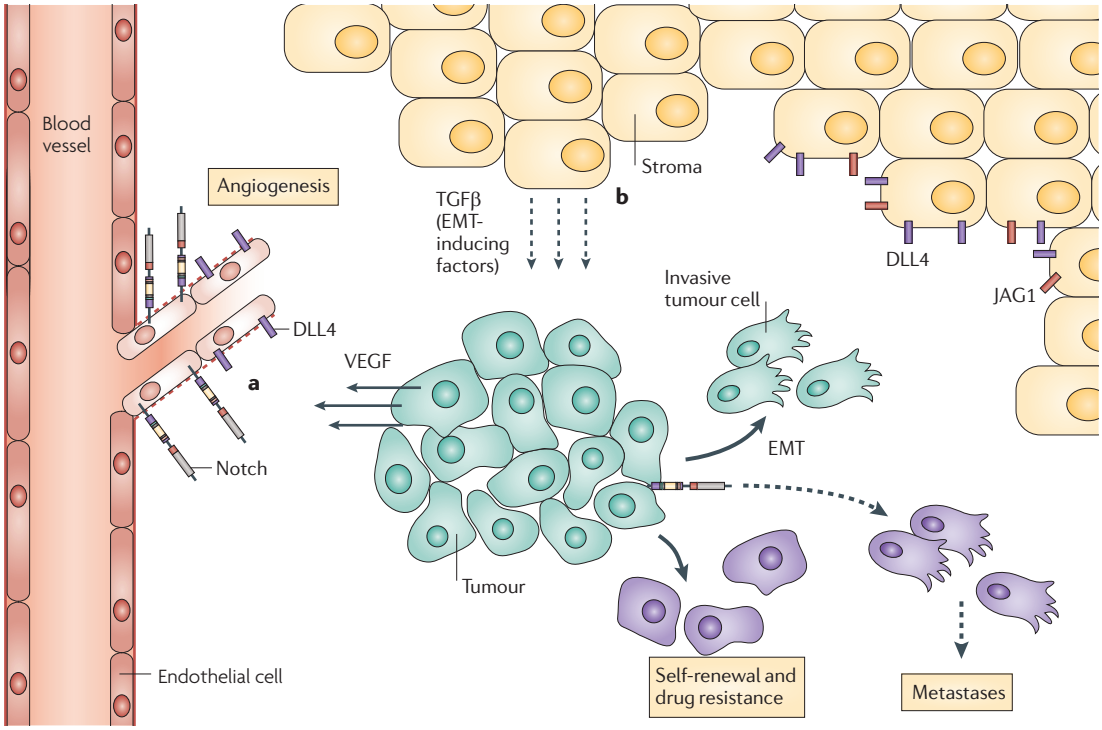

Figure 4 | **Notch-regulated tumour–microenvironment interactions in tumour maintenance and progression.** **a** | Notch signalling in angiogenesis is shown. The tumour secretes vascular endothelial growth factor (VEGF), inducing sprouting and branching of new vessels from existing blood vessels. Endothelial tip cells also increase their expression of Delta-like 4 (DLL4; purple) in response to VEGF. DLL4 then signals through Notch on adjacent endothelial tube cells to downregulate the expression of VEGF receptor 2 (VEGFR2) (not shown), leading to the inhibition of angiogenesis. **b** | Notch signalling in tumour self-renewal and metastasis is shown. The tumour receives cues from the stroma, including epithelial to mesenchymal transition (EMT)-inducing factors, such as transforming growth factor-β (TGFβ), in response to which the tumour cells acquire invasive (green invasive cells) or stem-like (purple cells) properties. Some of these cells may acquire both properties (purple invasive cells) (possibly owing to activated Notch signalling) and be able to metastasize and establish secondary tumours. Dashed arrows indicate poorly understood mechanisms.

to chemotherapeutic drugs[134]. In pancreatic cancer, the expression of nuclear NOTCH3 along with phospho-STAT3 and phospho-AKT is associated with an aggressive tumour phenotype[135]. Inhibiting the Notch pathway also sensitizes otherwise taxane-resistant colon cancer cells to mitotic arrest both *in vitro* and *in vivo*, suggesting that combining taxanes with a GSI could be a useful therapeutic strategy[136].

One mechanism for Notch-induced drug resistance that is evident in pancreatic tumour cell lines is the induction of the transcriptional repressor *HES1*, which downregulates PTEN in certain cell types[137]. Inhibition of the PI3K survival pathway with wortmannin or LY294002 results in reduced levels of the NICD in prostate cancer cells. This leads to loss of Notch-mediated p53 downregulation and thus sensitization to chemotherapeutic agents[138]. This is further supported by data showing that ectopic expression of NOTCH1 does not confer chemoresistance in cells treated with PI3K inhibitors[63]. Similar effects were observed by blocking mTOR (a kinase acting downstream of PI3K) with rapamycin, which prevents the inhibition of p53-mediated transcription by Notch, thus sensitizing the cells to drug treatment[63].

Notch-induced chemoresistance can also result from antagonism between Notch and EGFR, as observed in trastuzumab (Herceptin; Genentech)-resistant ERBB2-positive breast cancer. In these tumours, Notch signalling is inactive and the tumours are not sensitive to GSI treatment. However, treatment with trastuzumab or a dual-specificity RTK inhibitor that targets EGFR and ERBB2 induced the upregulation of Notch activity. Treatment with a combination of trastuzumab and a GSI induced apoptosis in these cells[139].

In oestrogen receptor (ER)-positive breast cancer cells, treatment with tamoxifen inhibits the response to oestrogen, but turns the Notch pathway on, leading to the activation of survival pathways. Notch interacts with ERα at the chromatin level and regulates a subset of ER-dependent genes. This crosstalk is probably dependent on the recruitment of IKKα to the chromatin by Notch, suggesting that IKKα could be a novel therapeutic target to specifically inhibit ER–Notch crosstalk[140]. Interestingly, an important role has been attributed to Notch in the maintenance of ER-negative tumours. These tumours show an increased expression of survivin, increased cell proliferation and reduced

apoptosis[141,142]. ER-negative tumours show reduced tumour growth when treated with a GSI, indicating a role for Notch pathway in the maintenance of these tumours[142].

A recent study by Wang *et al.*[143] has implicated the Notch pathway in the radioresistance of CSCs. This study demonstrated that inhibiting the Notch pathway with GSIs resulted in a reduction of AKT activity and made the glioma stem cells more radiosensitive[143]. Furthermore, combining GSIs with temozolomide (Temodar; Schering-Plough) treatment blocked the progression of brain tumours in 50% of the treated mice, which was probably due to blocking Notch in the CSCs and thus sensitizing them to drug treatment[144].

Taken together, these studies suggest that the activation of the Notch pathway can make tumour cells resistant to chemotherapy or radiation. A deeper understanding of the crosstalk between Notch and other signalling pathways will facilitate the design of novel therapeutic regimens that could sensitize tumour cells to chemotherapeutic agents and radiation.

Conclusions and future directions

In this Review we have discussed the evidence for a role of aberrant Notch signalling in solid tumours. As the title alludes to, we have found that Notch signalling in solid tumours seems to act in almost every tumorigenic process. Notch activity has been associated with the initiation and progression of neoplastic disease, and has been implicated in the maintenance of the neoplastic phenotype and resistance to therapeutic agents. Surprisingly though, there is little evidence to demonstrate that Notch signalling is constitutively activated through Notch gene mutations in these cancers. In fact, it seems to be likely that the hyperactivation of Notch receptors in tumours is through normal ligand-mediated events and/or loss of negative regulators and, therefore, remains sensitive to GSIs (BOX 3). In fact, there are at least four GSI compounds being evaluated for efficacy in the treatment of various tumours in nearly 20 ongoing clinical trials, which include trials in T-ALL, breast cancer, pancreatic cancer, glioblastoma and melanoma (see ClinicalTrials.gov; see Further information). Furthermore, several novel biological agents (such as, antibodies and decoys) are being developed to inhibit Notch signalling[145–147]. However, evidence also supports a context-dependent role for Notch as a tumour suppressor. Several lines of evidence that have been derived from mouse models suggest that the loss of *Notch1* can promote tumorigenesis. Although Notch itself does not fit the classical definition of a tumour suppressor, the loss of Notch activity can provide the proper environment to promote tumorigenesis in certain contexts. For example, it is possible that the loss of Notch activity could result in a change in cell fate to a cell type with greater proliferative capacity that may then be more prone to transformation.

What accounts for these pleiotropic effects that are governed by Notch signalling? Can we predict the outcome of Notch signalling in any given tumour? Perhaps Notch signalling in tumorigenesis represents a new paradigm in oncogenic signalling pathways. Unlike the 'classical' oncogenes such as RAS isoforms or BRAF, in which mutation renders activity constitutive in all cells, the Notch pathway seems to be inappropriately activated depending on cellular context. Moreover, not all Notch signalling is equal. Evidence suggests that the four Notch proteins have distinct activities and outcomes, although it is thought that the mechanistic details of action are similar. In fact, there is currently no clarity regarding specificity in Notch signalling with respect to each Notch protein. To compound this problem, recent evidence has suggested that distinct populations within a tumour can express distinct Notch paralogues. For example, in breast carcinoma the CSC population displayed NOTCH4 expression and activity, whereas the more differentiated cancer cells expressed NOTCH1 (REF. 148). Blocking NOTCH4 but not NOTCH1 by small interfering RNA negatively affects the CSCs[148]. Furthermore, evidence exists indicating that NOTCH2 can have a role in the progression of pancreatic carcinoma but that NOTCH1 cannot[149]. In fact, NOTCH1 may even have an opposing tumour suppressor function in pancreatic carcinoma[5]. If all four Notch proteins function in a mechanistically similar manner, how can these different activities be explained? Although much work will have to be done to answer these questions, it is intriguing to speculate that the different activities among the Notch proteins are primarily mediated by events on chromatin in the regulation of transcription. If we consider that the Notch–CSL–MAML1 core complex represents the initial scaffold on which a transcriptional regulatory complex is built, one can imagine that this is where the specificity lies. Certainly, we can hypothesize that, considering the milieu of transcriptional regulatory proteins, distinct Notch complexes can recruit or interact with a variety of factors that modulate the transcription of Notch target genes. Considering this concept, it becomes more evident how pathway crosstalk can influence Notch signalling outcome.

This then presents a problem in that many contextual cues via pathway crosstalk might determine the outcome of a cancer treatment that is based on the inhibition of Notch signalling. Thus, the barrier to effective combinatorial treatment regimens will be the elucidation of the relevant signalling networks that interface with Notch. Despite the wealth of studies investigating aspects of Notch signalling, the research field is still lacking the emergence of universal themes that would provide information about how Notch affects so many neoplasms and whether the inhibition of Notch signalling would prove to be a 'magic bullet' in cancer care. However, what we have discovered is that Notch is not the whole story, but merely the preface to a 'Tolstoyesque' epic. Research in the coming years should aim to decipher the complex crosstalk networks that are governed by Notch and that influence Notch signalling. Only then will we be able to effectively target the Notch pathway in cancer.

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