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Review

The Notch pathway in cancer: Differentiation gone awry

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Abstract

The Notch signalling cascade influences several key aspects of normal development by regulating differentiation, proliferation and apoptosis. Its association to human cancer is firmly established in T-cell leukaemia where point mutations or chromosomal translocations lead to constitutive signalling. Accumulating data indicate that deregulated Notch activity is involved also in the genesis of other human cancers, such as pancreatic cancer, medulloblastoma and mucoepidermoid carcinoma. In these tumours, the oncogenic effect of Notch signalling reflects an aberrant recapitulation of the highly tissue-specific function of the cascade during normal development and in tissue homeostasis.

Keywords: Notch; Cancer; Differentiation; T-ALL; Breast cancer

1. Introduction

1.1. The Notch signalling cascade: the core axis

In multicellular organisms cell–cell communication represents a very important aspect of cell fate decision. Since the first observation in the early twentieth century of a strain of the fruit fly *Drosophila melanogaster* with notches at their wingblade [1], the appreciation of the Notch cascade as one essential and highly complex aspect of juxtacrine signalling has steadily increased. For a long time this was a research field of interest mainly for developmental biologists working on cell fate determination in model organisms such as *D. melanogaster* and the nematode worm *Caenorhabditis elegans*. In these systems it was shown that Notch signalling was involved in regulating cell fate determination, proliferation and differentiation in a vast array of cells and tissues [2]. Depending on cell type, Notch can either inhibit or delay differentiation, but in some instances also induce a secondary fate selection. In addition, it can also affect important cellular processes such as proliferation and apoptosis.

A role in human tumour genesis became apparent when it was found that the Notch1 receptor gene was engaged in chromosomal translocations in a subset of T-cell acute lymphoblastic leukaemias (T-ALL) [3]. Deregulated Notch signalling appears however also to play a role in the genesis of other human malignancies. Similar to what has been shown in many other pathways involved in oncogenesis, the tumour-associated events might take place at several levels in the Notch cascade. In order to fully appreciate what molecular events that might lead to deregulated Notch signalling it is important to be familiar with the main components of the cascade. We would like to emphasize that the following brief summary of the Notch pathway only covers the basic aspects of its composition and function. During recent years numerous excellent reviews have been published which covers this in greater detail (see for example [4–7]).

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In mammals the core components of the cascade comprise ligands (Delta-like-1, -3, -4 and Jagged-1 and-2) and four transmembrane receptors (Notch1 to Notch4). The receptors share the same overall structure and are synthesized as single precursor proteins, which are cleaved into two associated peptides during transport to the cell surface [5,8]. Thus, the functional receptor is composed of an extracellular ligand-binding domain anchored to the membrane by its interaction with the transmembrane/cytoplasmic part of the receptor (Fig. 1). The extracellular domain comprises a set of epidermal growth factor (EGF)-like repeats responsible for ligand binding. The cytoplasmic domain harbours several structurally conserved motifs, including a RAM-domain involved in CSL-binding (see below) and six ankyrin repeats. Importantly, it also comprises two nuclear localization domains, a transcriptional activation domain and a PEST sequence [9]. The latter mediates a rapid degradation of the intracellular domain. Upon ligand binding, the receptor is subjected to two proteolytic events as part of the activation mechanism [10,11]. The first cleavage occurs in close proximity to the extracellular side of the plasma membrane followed by a second cleavage within the transmembrane domain, mediated by a γ-secretase complex, which leads to the release of the intracellular domain of the receptor (intracellular Notch, icN) from the membrane. Interestingly, the γ-secretase complex with its catalytic component presenilin has other cellular substrates including the β-amyloid precursor protein, and aberrant processing of this protein is implicated in Alzheimer's disease [12]. This fact has led to development of numerous commercially available pharmacological inhibitors of γ-secretase activity, which has proven very valuable also in research on Notch signalling. Upon cleavage, the icN translocates to the nucleus where it forms a complex with the ubiquitously expressed transcription factor CSL (CBF1/Su(H)/LAG-1, also known as RBP-Jκ) . In the absence of icN, CSL is a transcriptional repressor due to its association with co-repressors (Fig. 1) [13–15]. When icN associates with CSL, a number of co-activators are recruited including mastermind-like (MAML-1, -2, and -3) resulting in a multiprotein complex, which acts as a potent transcriptional activator [16–18]. The transcriptional targets of Notch signalling include in particular differentiation related factors but also cell cycle regulators (p21 and cyclin D1) and regulators of apoptosis (for review see [2,19]). Transcription factors of the Hairy/enhancer of split (Hes) and Hes related (HRT/HRP/Hey) families are the most well-

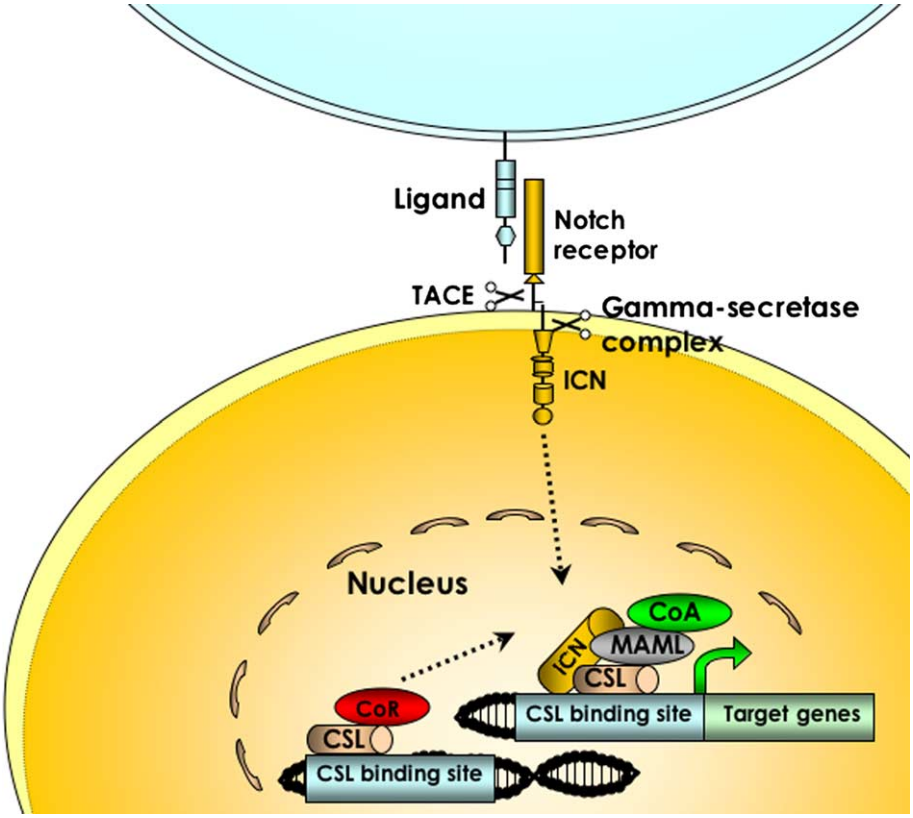

Fig. 1. The core axis of Notch signalling. Notch signalling is initiated by ligand–receptor interaction between adjacent cells. Interaction results in two consecutive cleavages of the receptor, which are executed by a metalloproteinase (TACE) and a γ-secretase complex, subsequently leading to the release of the intracellular domain Notch (icN). Intracellular Notch translocates into the nucleus and binds the CSL transcription factor, thereby displacing co-repressors (CoR) and recruiting co-activators (CoA), such as MAML proteins, where after transcription is initiated. Downstream targets include members of Hes and Hes related bHLH repressor families.

known direct target genes of CSL [20]. These proteins belong to the basic helix-loop-helix family of transcription factors and bind specific sequences in the promoter region of target genes and repress the transcription through recruitment of a set of co-repressors including the Groucho/TLE protein. Recent data indicate however that, specific signalling pathways can modulate cofactor recruitment, switching Hes1 mediated repression to activation [21].

It is important to point out that Notch signalling can be modulated by numerous proteins that affect the activity of the cascade [6]. In addition, Notch signalling can diverge at several points along this core axis described in this brief summary, i.e., there are CSL-independent effects of icN and there are other target genes for icN in complex with CSL than Hes and Hes related factors. When analyzing the activity of the Notch cascade, for example in analyses of tumour material, the expression level of Hes1 is often used as a surrogate marker of Notch signalling activity.

In the prototypical function of the pathway, in for example neuronal cells, Hes and Hes related proteins repress the expression of differentiation promoting bHLH factors thereby maintaining the cells in an immature state [22]. It is however becoming evident that Notch signalling not only restricts differentiation along a specific pathway but also take an active part in directing differentiation towards an alternate fate. In order to understand the role of deregulated Notch signalling in various tumour forms, it is therefore of fundamental importance to delineate the function of the cascade in the specific tissue from which the tumours are derived.

2. Genetic alterations of Notch receptors in cancer

2.1. T-cell acute lymphoblastic leukaemia

The characterization of chromosomal translocations in human malignancies has for many years served as a rich source for identifying oncogenes. With the finding that the t(7;9)(q34;q34.3) translocation in T-ALL led to the juxtaposition of *Notch1* with the *T-cell receptor beta (TCR-β)*, the oncogenic properties of Notch1 became apparent (Fig. 2) [3]. The translocation, which arises due to mistakes during the TCR recombination process, leads to expression of icN1 in a TCR-β regulated manner. Thus, the signalling activity of this truncated form of icN1 is independent of ligand stimulation and hence refractory to normal regulation. The oncogenic effect of deregulated Notch activity in T-cells is intimately linked to the function of Notch signalling during several stages of normal T-cell differentiation [23]. In early lymphoid commitment Notch activity promotes T-cell fate over the default B-cell lineage, but a regulated activity of the Notch cascade is also important during later stages of T-cell differentiation [24–26]. In particular, Notch signalling is down regulated in the

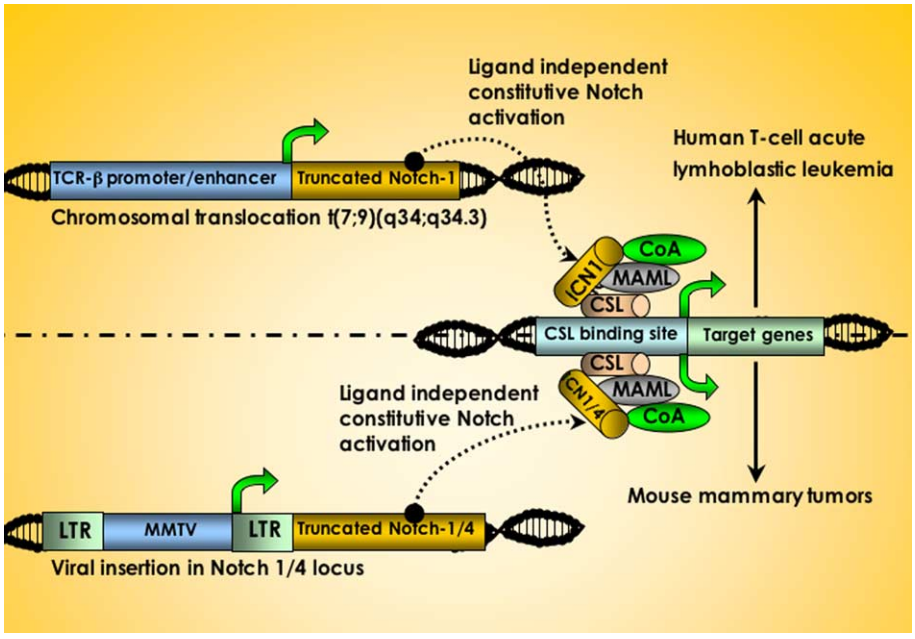

Fig. 2. Two examples of genetic alterations at the receptor level. In a subset of T-ALL, a chromosomal translocation leads to juxtaposition of a truncated *Notch1* gene with the *TCR*-β promoter/enhancer. This results in ligand-independent, constitutive activation of icN1 and deregulation of target genes. In mouse mammary tumours, mouse mammary tumour virus (MMTV) insertions can take place in the *Notch1* or *Notch4* genes. In analogy with the t(7;9) translocation in T-ALL, this leads to constitutive expression of Notch1 or Notch4 intracellular domains and development of mammary tumours.

double positive (DP, $CD4^+$ $CD8^+$) stage and studies in mouse model systems show that enforced expression of icN1 leads to accumulation of cells at this stage [27]. It is therefore likely that the oncogenic effect of icN1 in T-cells is associated with both its capacity to promote T cell commitment and thereafter to block differentiation at the DP stage. The combined effect of these events is an expanded pool of DP T-cells with an increased risk of accumulating additional mutations. It should be stressed that the t(7;9) translocation is exceedingly rare in T-ALL, but nevertheless accumulating data implicate a key role for deregulated Notch activity in T-ALL. Notch3 is expressed in a vast majority of T-ALL and might be one reason for elevated Notch signalling in T-ALL [28]. Recent findings indicate however that Notch1 deregulation might be a much more frequent event in T-ALL than previously appreciated. In a very interesting study from Weng and colleagues it was reported that a substantial portion of t(7;9) negative T-ALLs were growth-suppressed upon treatment with γ-secretase inhibitors. This observation instigated a search for mutations in the *Notch1* gene in these tumours, and unexpectedly they found frequent point mutations in the region of the Notch1 receptor responsible for heterodimerisation of the S1 cleaved functional receptor [29]. They also detected mutations in the PEST domain of icN1, which led to expression of a truncated form of the protein. In primary T-ALL, these mutations were present in more than half of the tumours (56%). Importantly, no such mutations were detected in B-ALLs emphasizing the specific oncogenic function of Notch1 in the T-cell lineage. The functional consequences of these mutations are not entirely clarified, but data presented indicate that mutations in the heterodimerisation domain of the receptor might make it more susceptible to ligand-independent γ-secretase activity while truncation of the C-terminal PEST domain would increase the half-life of icN1 (Fig. 3). These findings are important in several ways for the understanding of T-cell leukemogenesis. Firstly, deregulated Notch-receptor function is a much more common oncogenic event in T-ALL than previously appreciated. Secondly, some of the tumours with Notch1 mutations misexpress other oncogenes associated with T-ALL, such as *Hox11* and *Tal-1,* which indicates that these genes might cooperate with deregulated Notch signalling in T-ALL. Thirdly, the strong selection for Notch deregulation in T-ALL indicates that Notch directed therapies might be applied in a large proportion of T-ALLs [29]. In summary, deregulated Notch1 seems to be a very important aspect of T-ALL since mutations in the Notch1 receptor (and hypersignalling activity) are present in more than half of the tumours. The oncogenic function of the Notch cascade in T-cells is associated with its role in normal T-cell development and when illegitimate Notch activation occurs the differentiation process is halted, and immature cells vulnerable for additional oncogenic mutations accumulate.

2.2. Breast cancer

In mice, slowly transforming mouse retroviruses have proven a very useful tool for identifying oncogenes. These viruses, which themselves do not express transforming

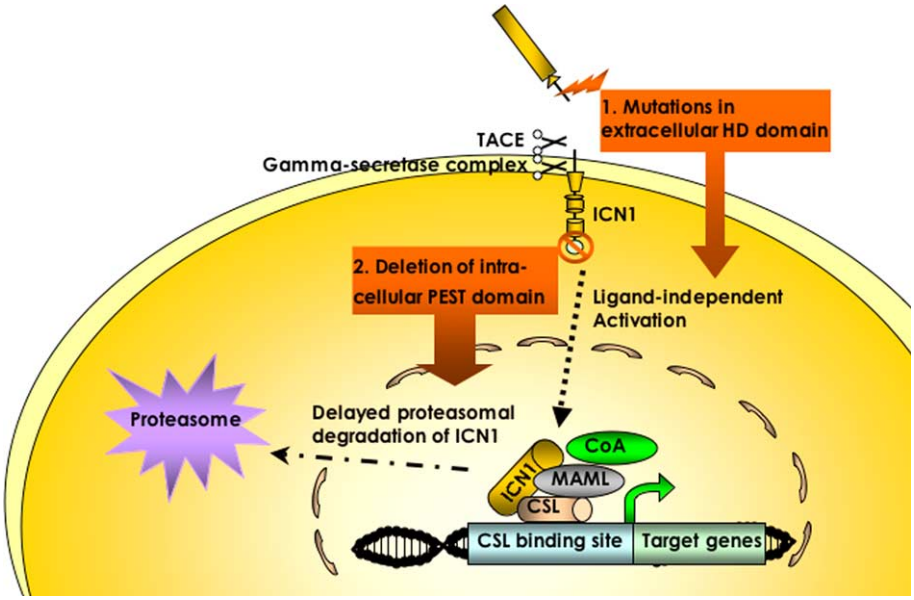

Fig. 3. Functional consequences of activating mutations of Notch1 detected in T-ALL. The mutations found in the heterodimerisation domain (HD) are expected to cause ligand-independent activating cleavages of Notch1, and release of intracellular Notch1 (icN1). PEST domain mutations are believed to result in stabilisation of the CSL/icN1/MAML transcriptional complex due to delayed proteasomal degradation of icN1, leading to augmented activation of target genes.

protein products integrate as part of their life cycle into the mouse genome. If this insertion takes place in the vicinity of a proto-oncogene, the strong enhancer of the virus causes constitutive activation of the flanking gene, which initiates the malignant transformation [30]. By using the mouse mammary tumour virus (MMTV) it was shown that *Notch4* (also known as *int-3*) was frequently targeted by the virus leading to tumours in the mouse mammary [31]. The viral insertion resulted in the constitutive expression of a small portion of the extracellular domain and the entire transmembrane/intracellular domain of the Notch4 receptor (Fig. 2). The oncogenic potential of this truncated form of Notch4 in epithelial cells has been confirmed both *in vitro* and *in vivo* using experimental model systems [32–36]. As in the case of T-ALL, the oncogenic effect of Notch activation can be attributed to blocked differentiation of mammary epithelial cells, though the information regarding the role of Notch signalling in normal mammary epithelial differentiation remains relatively sparse. The Notch pathway has also been linked to genetic events strongly associated with breast cancer development. For example, the expression of the growth promoting receptor tyrosine kinase erbB2 is induced in a CSL-dependent manner [37]. In addition, in a subset of transgenic mice expressing erbB2 infected with MMTV, the *Notch1* gene was targeted, indicative of a functional cooperativity between the erbB2 and Notch signalling pathways [38]. Another Notch target gene of potential interest for the oncogenic function of Notch in mammary epithelial cells is cyclin D1 [39,40]. Recently, Artavanis-Tsakonas and co-workers developed a transgenic mouse model in which the icN1 was under the control of the MMTV promoter [41]. These mice developed lactation dependent neoplasms. In addition, they also developed mice transgenic for the Notch inhibitor Deltex. When these mice, in which Notch signalling is specifically inhibited in mammary epithelial cells, were crossed with the established MMTV-Hras1 mice, which are prone to develop cyclin D1 dependent mammary tumours, tumour formation was attenuated and cyclin D1 levels were low in the mammary epithelium. These findings suggest that Notch activation might mediate certain aspects of the Ras-induced tumourigenesis in mouse mammary epithelium.

In humans, the evidences for a deregulated Notch cascade in breast cancer remains sparse. However, a correlation between Ras overexpression and elevated Notch1 levels has been reported [42]. Furthermore, in a recent paper it was shown that expression of Numb, a negative regulator of Notch signalling, was lost in approximately 50% of primary human mammary carcinomas and that these tumours showed increased Notch1 activity [43]. In addition, Numb negative breast tumour cells were sensitive to pharmacological inhibition of Notch. Thus, in mouse mammary epithelium, deregulated Notch activity is oncogenic, and recent studies indicate that this might be the case also in human breast cancer, but further studies are required to clarify these matters.

2.3. Medulloblastoma

In an analysis of embryonal brain tumours, medulloblastomas or primitive neuroectodermal tumours, it was shown that *Notch2* was amplified in 15% of the cases, leading to elevated Notch2 levels [44]. Furthermore, when analyzing Hes1 as a surrogate marker for Notch activity it was found that elevated expression was associated with decreased survival probability. Interestingly, during normal development Notch2 expression is high in proliferating cerebellar granule progenitor cells, while Notch1 is expressed in post-mitotic differentiating cells. Experimental evidence further supports a scenario in which Notch1 and Notch2 have opposite effects in medulloblastoma cells, with the latter supporting growth and hence transformation while the former is associated with a non-proliferating, differentiating cell type. Importantly, the oncogenic properties of the two receptors reflect the disparate functions of Notch1 and Notch2 during development of the cerebellum. Further studies are needed to explain the different effects of Notch1 and Notch2 signalling in medulloblastoma, but underlines the notion that it is imperative to understand the normal function of the cascade in the tissue from which the tumour arise in order to fully appreciate its role in tumourigenesis. Somewhat contradictory, another study indicate that a majority of human medulloblastomas expressed elevated Notch1 levels while Notch2 expression was elevated only in a subset of analyzed tumours [45]. Thus, additional analyses are needed to clarify this discrepancy, but taken together both studies indicate that deregulated Notch activity might play a previously unappreciated role in the development of human medulloblastomas.

3. Short-circuiting the pathway at the intracellular level

3.1. Mucoepidermoid carcinoma

Mucoepidermoid carcinoma (MEC) is a tumour that arises in the salivary glands of the upper aerodigestive tract. Cytogenetic analyses of this relatively rare tumour showed that a t(11;19)(q14–21;p12–13) translocation was associated with the disease [46–48]. When the breakpoint subsequently was cloned it turned out to involve the *MECT1* and *MAML2* genes [49]. As pointed out earlier, MAML interacts with icN and is instrumental for the formation of the multiprotein complex that converts CSL from a repressor to an activator [50]. As a consequence of the t(11;19) translocation a tumour-specific fusion protein is expressed, containing a short

piece of the MECT1 protein and a large piece of the MAML2 protein (Fig. 4) [49]. Importantly, the expression of the fusion protein is under control of the *MECT1* promoter, thereby disrupting the normal regulatory control of MAML2 expression in the tumour cells. The transforming capacity of the MECT1–MAML2 fusion protein was also confirmed in foci formation assays. Despite the finding that several Notch target genes, such as *Hes1* and *Hey1* are elevated in MEC cell lines the mechanism behind this activation remains obscure. For example, experimental data suggest that the fusion protein can inhibit the CSL dependent induction of *Hes1* promoter activity. Thus, the expression of MECT–MAML fusion protein disrupts conventional Notch signalling and activates Notch target gene expression in an icN- and CSL-independent fashion through mechanisms that have to be studied further.

3.2. Notch and viruses

Given the importance of Notch signalling in many key cellular processes it is not entirely surprising that several viruses plug into the Notch signalling pathway to exploit host cell functions in a way that is beneficial for the virus' life cycle [51]. The Epstein Barr virus (EBV) is a herpesvirus associated with lymphoid and epithelial cell tumours. The virus is normally non-pathogenic and over 90% of adults are EBV positive. In immunodeficient patients the normally small pool of latently infected B-cells can expand and give rise to B-cell lymphoproliferative disorders [52]. Several groups discovered that one of the virus proteins, EBNA2, which is involved in up regulation of proteins involved in maintaining latency, could interact with CSL [53–55]. EBNA2 binds to the repressor domain of the CSL protein and displace the co-repressor complex, converting CSL from a transcriptional repressor to an activator, thereby mimicking the effect of icN [56]. Also other proteins of the EBV (EBNA-3a and -3b) can interact with the same domain of CSL, but with opposite effect [57,58]. Thus, through the action of several viral proteins EBV deregulates the Notch cascade, thereby influencing the host cell to create an environment beneficial to the virus. Furthermore, in the human papillomavirus (HPV) mediated cervical carcinoma perturbation of Notch signalling seems to be an integral part of disease progression, and it should be noted that the HPV-encoded E6 protein can associate with MAML (for review [59]).

4. Guilt by association: overexpression of Notch cascade components in tumours

Overexpression of various proteins in the Notch signalling cascade has been found in a number of different tumours, such as renal cell carcinoma, prostate cancer, multiple myeloma and Hodgkin's and anaplastic lymphoma [60–64]. The causative link between elevated expression and the tumourigenic process in these tumours remains however to be fully explored.

In an interesting paper by Miyamoto and colleagues [65], it was shown that the Notch cascade might be an important mediator of pre-neoplastic lesions in pancreatic cancer. In primary pancreatic cancers the Notch pathway components, including ligands, receptors and downstream target genes were up regulated compared to normal pancreas. Importantly, Notch signalling plays a central role in cell fate decision during embryonic development of the pancreas, where it maintains an undifferentiated precursor cell type [66–68]. Therefore it was suggested that elevated Notch signalling in pancreatic cancer leads to accumulation of undifferentiated precursor cells. The mechanistic explanation for elevated Notch signalling in this tumour form might be

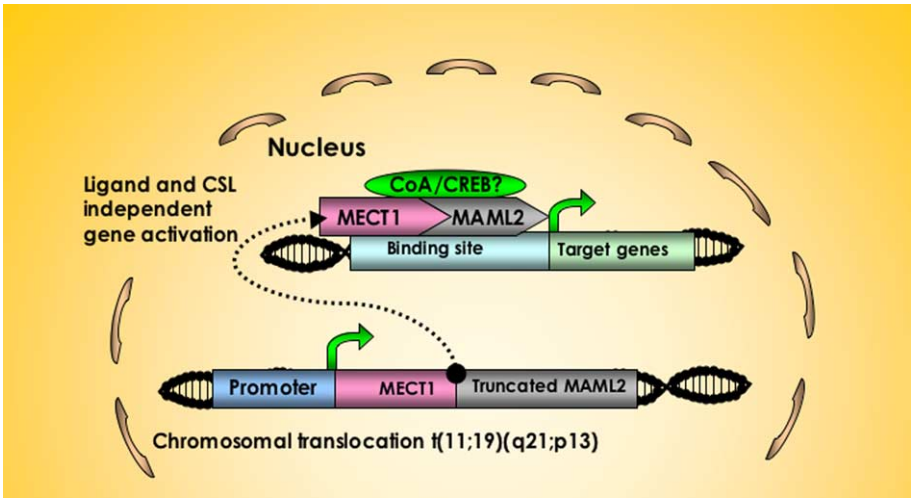

Fig. 4. The t(11;19) translocation found in mucoepidermoid carcinoma, results in expression of MECT1–MAML2 fusion proteins. This fusion protein appears to be able to recruit co-activators (CoA) and activate Notch target genes in a CSL- and ligand-independent fashion.

associated with activation of the Ras-pathway (either through direct Ras mutations or through transforming growth factor-α (TGF-α) induced EGFR/Ras/MAPK activation), which almost invariably is found in pancreatic cancer [69]. The authors show that important aspects of Ras-pathway function seems to be mediated by augmented Notch signalling in pancreatic cells and propose that elevated Ras/MAPK signalling through the EGFR in exocrine pancreas leads to Notch activation, which in turn leads to dedifferentiation of exocrine cells. These immature cells form pre-neoplastic lesions susceptible for additional mutations, which eventually might lead to the formation of invasive ductal cancer. Furthermore, accumulating data indicate that the cross-talk between the Ras/MAPK and Notch cascades is at hand also in other cell systems [42,70–74].

In our group, we have studied the role of Notch signalling in human neuroblastoma cells. This childhood tumour, which originates from the sympathetic nervous system, is characterized by a perturbed differentiation [75]. During induced differentiation of neuroblastoma cells there was a transient induction of Notch1 and Hes1, and a down regulation of the Hes1 target gene *Hash1* [76–78]. When the cells reached terminal differentiation the Notch signalling cascade was down regulated. Furthermore, introduction of icN1 inhibited this induced differentiation, indicating that regulated Notch signalling might be an integral part of neuroblastoma cell differentiation. When neuroblastoma cells were exposed to low oxygen tension, hypoxia, we noticed a down regulation of neuronal marker genes and up regulation of genes associated with neural crest functions, suggesting that hypoxic neuroblastoma cells adopt a more immature and hence more malignant phenotype [79,80]. Of particular importance, the hypoxic adaptation was associated with a persistent up regulation of several components of the Notch signalling cascade, which might play a role in the induction of an immature phenotype. If induction of Notch signalling by hypoxia also occurs in other tumour cell types, it might represent an important aspect of hypoxia-driven heterogeneity and aggressiveness. In addition, Dlk-1, which is a Delta homologue implicated in sympathoadrenal differentiation, is highly expressed in a subset of neuroblastomas [81]. A recent study showed that elevated Dlk-1 expression serves as a strong prognostic marker for adverse disease [82]. Albeit the functional role of Dlk1 in Notch signalling remains obscure, these findings further implies a role for this cascade in the genesis of neuroblastoma.

5. Turning the table: Notch as a tumour suppressor

It has been stressed throughout this article that the consequence of Notch activity is highly context dependent and that any involvement of the cascade in cancer has to relate to this normal function in a given tissue. In most tissues, active Notch signalling is associated with an immature cell type and for terminal differentiation to occur, this signalling activity has to be down regulated. However, in some tissues the situation is the opposite, i.e., Notch signalling induces differentiation. In these tissues, loss of Notch signalling activity might be associated with blocked differentiation and hence tumour progression.

The most clear cut evidence that loss of Notch signalling is oncogenic and has properties of a tumour suppressor, stems from studies of Notch function in mouse skin. Here, Notch signalling seems to be involved in inducing differentiation, as indicated by induction of early differentiation markers and the cell cycle inhibitor p21 [83]. Importantly, tissue specific ablation of Notch1 in mouse epidermis not only resulted in hyperplastic growth followed by development of basal cell carcinomas, but also enhanced chemically induced skin carcinogenesis [84]. These effects might be associated with Notch associated perturbation of the Wnt and Sonic hedgehog signalling pathways, which are strongly associated with skin cancer development. Whether the Notch pathway has similar functions in human skin cancer awaits further studies.

In the developing lung, Notch activation inhibits development of neuroendocrine cells and promotes epithelial growth [85]. In small cell lung cancer, which is of neuroendocrine origin, Notch signalling is not active and the differentiation related factor *Hash1*, a target gene for Hes1, is expressed [86,87]. Consequently, introduction of icN1 caused growth arrest in the latter cell type, characterized by elevated levels of p21 [88]. In contrast, in non-small cell lung cancer the Notch signalling cascade is active, leading to high expression levels of Notch target genes, such as *Hes1* and *Hey1* [86]. As a result, this tumour type might be susceptible to therapies based on Notch inhibition. In summary, lung cancer represents an interesting tumour entity where the activation of Notch can infer either tumour promotion or growth inhibition, depending on tumour cell type.

6. Therapeutic implications: problems and possibilities

Mounting evidence indicates that perturbation of Notch signalling might be involved in the genesis of many different tumour forms, and it is therefore of great potential importance to develop strategies to modulate the activity of the cascade as a novel treatment modality. The principal approaches could involve interference at any level of the cascade, i.e., by blocking ligand binding; by inhibition of intramembranous cleavage; or by interfering with the action of icN.

The principle of blocking ligand–receptor interaction has for example been successfully tested in the context of

Notch-induced adipocyte differentiation, using recombinant EGF repeats [89]. Inhibition of receptor activity is readily achieved using chemical inhibitors of the γ-secretase activity. Since all Notch receptors are dependent on this proteolytic event, inhibition of γ-secretase thus target the activation step of all Notch receptor types expressed in a given tumour. Since γ-secretase inhibition also affects other targets, such as APP, it will be very important to develop selective Notch inhibitors and such drugs are underway [12,90]. In light of the recent finding that activating, γ-secretase-inhibition sensitive, *Notch1* mutations are prevalent in T-ALL, this approach is particularly appealing for treatment of this disease. Alternatively, it might be possible to interfere with the function of icN [16]. Recently published data have greatly extended our understanding of the multiprotein complex that is formed with CSL upon interaction with icN1. This knowledge could potentially be applied when designing small inhibitory molecules that exclusively block Notch signalling at this level. Since Notch in some tissues might function as a tumour suppressor gene it seems very important to specifically target the tumour tissue, an outstanding challenge shared with other cancer treatment strategies.

Conflict of interest statement

None declared.

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References

1. Morgan T. The theory of the gene. *Am Nat* 1917, **51**, 513–544.
2. Artavanis-Tsakonas S, Rand MD, Lake RJ. Notch signaling: cell fate control and signal integration in development. *Science* 1999, **284**(5415), 770–776.
3. 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**(4), 649–661.
4. Lai EC. Notch signaling: control of cell communication and cell fate. *Development* 2004, **131**(5), 965–973.
5. Mumm JS, Kopan R. Notch signaling: from the outside in. *Dev Biol* 2000, **228**(2), 151–165.
6. Kadesch T. Notch signaling: the demise of elegant simplicity. *Curr Opin Genet Dev* 2004, **14**(5), 506–512.
7. Hansson EM, Lendahl U, Chapman G. Notch signaling in development and disease. *Semin Cancer Biol* 2004, **14**(5), 320–328.
8. Logeat F, Bessia C, Brou C, *et al*. The Notch1 receptor is cleaved constitutively by a furin-like convertase. *Proc Natl Acad Sci USA* 1998, **95**(14), 8108–8112.
9. Gupta-Rossi N, Le Bail O, Gonen H, *et al*. Functional interaction between SEL-10, an F-box protein, and the nuclear form of activated Notch1 receptor. *J Biol Chem* 2001, **276**(37), 34371–34378.
10. Brou C, Logeat F, Gupta N, *et al*. A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell* 2000, **5**(2), 207–216.
11. Mumm JS, Schroeter EH, Saxena MT, *et al*. A ligand-induced extracellular cleavage regulates γ-secretase-like proteolytic activation of Notch1. *Mol Cell* 2000, **5**(2), 197–206.
12. Fortini ME. Gamma-secretase-mediated proteolysis in cell-surface-receptor signalling. *Nat Rev Mol Cell Biol* 2002, **3**(9), 673–684.
13. Hsieh JJ, Zhou S, Chen L, *et al*. CIR, a co-repressor linking the DNA binding factor CBF1 to the histone deacetylase complex. *Proc Natl Acad Sci USA* 1999, **96**(1), 23–28.
14. Jarriault S, Brou C, Logeat F, *et al*. Signalling downstream of activated mammalian Notch. *Nature* 1995, **377**(6547), 355–358.
15. Kao HY, Ordentlich P, Koyano-Nakagawa N, *et al*. A histone deacetylase co-repressor complex regulates the Notch signal transduction pathway. *Genes Dev* 1998, **12**(15), 2269–2277.
16. Fryer CJ, White JB, Jones KA. Mastermind recruits CycC:CDK8 to phosphorylate the Notch ICD and coordinate activation with turnover. *Mol Cell* 2004, **16**(4), 509–520.
17. Wu L, Aster JC, Blacklow SC, *et al*. MAML1, a human homologue of Drosophila mastermind, is a transcriptional co-activator for NOTCH receptors. *Nat Genet* 2000, **26**(4), 484–489.
18. Wu L, Sun T, Kobayashi K, *et al*. Identification of a family of mastermind-like transcriptional co-activators for mammalian notch receptors. *Mol Cell Biol* 2002, **22**(21), 7688–7700.
19. Nickoloff BJ, Osborne BA, Miele L. Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. *Oncogene* 2003, **22**(42), 6598–6608.
20. Iso T, Kedes L, Hamamori Y. HES and HERP families: multiple effectors of the Notch signaling pathway. *J Cell Physiol* 2003, **194**(3), 237–255.
21. Ju BG, Solum D, Song EJ, *et al*. Activating the PARP-1 sensor component of the groucho/ TLE1 co-repressor complex mediates a CaMKinase IIdelta-dependent neurogenic gene activation pathway. *Cell* 2004, **119**(6), 815–829.
22. de la Pompa JL, Wakeham A, Correia KM, *et al*. Conservation of the Notch signalling pathway in mammalian neurogenesis. *Development* 1997, **124**(6), 1139–1148.
23. Pear WS, Aster JC. T cell acute lymphoblastic leukemia/lymphoma: a human cancer commonly associated with aberrant NOTCH1 signaling. *Curr Opin Hematol* 2004, **11**(6), 426–433.
24. Pui JC, Allman D, Xu L, *et al*. Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* 1999, **11**(3), 299–308.
25. Radtke F, Wilson A, Stark G, *et al*. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* 1999, **10**(5), 547–558.
26. Ciofani M, Schmitt TM, Ciofani A, *et al*. Obligatory role for cooperative signaling by pre-TCR and Notch during thymocyte differentiation. *J Immunol* 2004, **172**(9), 5230–5239.
27. Allman D, Karnell FG, Punt JA, *et al*. Separation of Notch1 promoted lineage commitment and expansion/transformation in developing T cells. *J Exp Med* 2001, **194**(1), 99–106.
28. Screpanti I, Bellavia D, Campese AF, *et al*. Notch, a unifying target in T-cell acute lymphoblastic leukemia?. *Trends Mol Med* 2003, **9**(1), 30–35.
29. Weng AP, Ferrando AA, Lee W, *et al*. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 2004, **306**(5694), 269–271.

30. Mikkers H, Berns A. Retroviral insertional mutagenesis: tagging cancer pathways. *Adv Cancer Res* 2003, **88**, 53–99.
31. Gallahan D, Callahan R. Mammary tumorigenesis in feral mice: identification of a new int locus in mouse mammary tumor virus (Czech II)-induced mammary tumors. *J Virol* 1987, **61**(1), 66–74.
32. 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**(3), 345–355.
33. Gallahan D, Jhappan C, Robinson G, *et al*. Expression of a truncated Int3 gene in developing secretory mammary epithelium specifically retards lobular differentiation resulting in tumorigenesis. *Cancer Res* 1996, **56**(8), 1775–1785.
34. Robbins J, Blondel BJ, Gallahan D, *et al*. Mouse mammary tumor gene int-3: a member of the notch gene family transforms mammary epithelial cells. *J Virol* 1992, **66**(4), 2594–2599.
35. Uyttendaele H, Marazzi G, Wu G, *et al*. Notch4/int-3, a mammary proto-oncogene, is an endothelial cell-specific mammalian Notch gene. *Development* 1996, **122**(7), 2251–2259.
36. Soriano JV, Uyttendaele H, Kitajewski J, *et al*. Expression of an activated Notch4(int-3) oncoprotein disrupts morphogenesis and induces an invasive phenotype in mammary epithelial cells in vitro. *Int J Cancer* 2000, **86**(5), 652–659.
37. Chen Y, Fischer WH, Gill GN. Regulation of the ERBB-2 promoter by RBPJkappa and NOTCH. *J Biol Chem* 1997, **272**(22), 14110–14114.
38. Dievart A, Beaulieu N, Jolicoeur P. Involvement of Notch1 in the development of mouse mammary tumors. *Oncogene* 1999, **18**(44), 5973–5981.
39. Ronchini C, Capobianco AJ. Induction of cyclin D1 transcription and CDK2 activity by Notch (ic): implication for cell cycle disruption in transformation by Notch (ic). *Mol Cell Biol* 2001, **21**(17), 5925–5934.
40. Wang TC, Cardiff RD, Zukerberg L, *et al*. Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice. *Nature* 1994, **369**(6482), 669–671.
41. Kiaris H, Politi K, Grimm LM, *et al*. Modulation of notch signaling elicits signature tumors and inhibits hras1-induced oncogenesis in the mouse mammary epithelium. *Am J Pathol* 2004, **165**(2), 695–705.
42. Weijzen S, Rizzo P, Braid M, *et al*. Activation of Notch-1 signaling maintains the neoplastic phenotype in human Ras-transformed cells. *Nat Med* 2002, **8**(9), 979–986.
43. Pece S, Serresi M, Santolini E, *et al*. Loss of negative regulation by Numb over Notch is relevant to human breast carcinogenesis. *J Cell Biol* 2004, **167**(2), 215–221.
44. Fan X, Mikolaenko I, Elhassan I, *et al*. Notch1 and notch2 have opposite effects on embryonal brain tumor growth. *Cancer Res* 2004, **64**(21), 7787–7793.
45. Hallahan AR, Pritchard JI, Hansen S, *et al*. The SmoA1 mouse model reveals that notch signaling is critical for the growth and survival of sonic hedgehog-induced medulloblastomas. *Cancer Res* 2004, **64**(21), 7794–7800.
46. Dahlenfors R, Wedell B, Rundrantz H, *et al*. Translocation(11;19)(q14-21;p12) in a parotid mucoepidermoid carcinoma of a child. *Cancer Genet Cytogenet* 1995, **79**(2), 188.
47. Johansson M, Mandahl N, Johansson L, *et al*. Translocation 11;19 in a mucoepidermoid tumor of the lung. *Cancer Genet Cytogenet* 1995, **80**(1), 85–86.
48. Horsman DE, Berean K, Durham JS. Translocation (11;19)(q21;p13.1) in mucoepidermoid carcinoma of salivary gland. *Cancer Genet Cytogenet* 1995, **80**(2), 165–166.
49. Tonon G, Modi S, Wu L, *et al*. t (11;19) (q21;p13) translocation in mucoepidermoid carcinoma creates a novel fusion product that disrupts a Notch signaling pathway. *Nat Genet* 2003, **33**(2), 208–213.
50. Jeffries S, Robbins DJ, Capobianco AJ. Characterization of a high-molecular-weight Notch complex in the nucleus of Notch (ic)-transformed RKE cells and in a human T-cell leukemia cell line. *Mol Cell Biol* 2002, **22**(11), 3927–3941.
51. Hayward SD. Viral interactions with the Notch pathway. *Semin Cancer Biol* 2004, **14**(5), 387–396.
52. Thorley-Lawson DA. Epstein–Barr virus: exploiting the immune system. *Nat Rev Immunol* 2001, **1**(1), 75–82.
53. Henkel T, Ling PD, Hayward SD, *et al*. Mediation of Epstein–Barr virus EBNA2 transactivation by recombination signal-binding protein J kappa. *Science* 1994, **265**(5168), 92–95.
54. Waltzer L, Logeat F, Brou C, *et al*. The human J kappa recombination signal sequence binding protein (RBP-J kappa) targets the Epstein–Barr virus EBNA2 protein to its DNA responsive elements. *Embo J* 1994, **13**(23), 5633–5638.
55. Grossman SR, Johannsen E, Tong X, *et al*. The Epstein–Barr virus nuclear antigen 2 transactivator is directed to response elements by the J kappa recombination signal binding protein. *Proc Natl Acad Sci USA* 1994, **91**(16), 7568–7572.
56. Hsieh JJ, Hayward SD. Masking of the CBF1/RBPJ kappa transcriptional repression domain by Epstein–Barr virus EBNA2. *Science* 1995, **268**(5210), 560–563.
57. Cooper A, Johannsen E, Maruo S, *et al*. EBNA3A association with RBP-Jkappa down-regulates c-myc and Epstein–Barr virus-transformed lymphoblast growth. *J Virol* 2003, **77**(2), 999–1010.
58. Johannsen E, Miller CL, Grossman SR, *et al*. EBNA-2 and EBNA-3C extensively and mutually exclusively associate with RBPJkappa in Epstein–Barr virus-transformed B lymphocytes. *J Virol* 1996, **70**(6), 4179–4183.
59. Lefort K, Dotto GP. Notch signaling in the integrated control of keratinocyte growth/differentiation and tumor suppression. *Semin Cancer Biol* 2004, **14**(5), 374–386.
60. Rae FK, Stephenson SA, Nicol DL, *et al*. Novel association of a diverse range of genes with renal cell carcinoma as identified by differential display. *Int J Cancer* 2000, **88**(5), 726–732.
61. Santagata S, Demichelis F, Riva A, *et al*. JAGGED1 expression is associated with prostate cancer metastasis and recurrence. *Cancer Res* 2004, **64**(19), 6854–6857.
62. Houde C, Li Y, Song L, *et al*. Overexpression of the NOTCH ligand JAG2 in malignant plasma cells from multiple myeloma patients and cell lines. *Blood* 2004, **104**(12), 3697–3704.
63. Jundt F, Anagnostopoulos I, Forster R, *et al*. Activated Notch1 signaling promotes tumor cell proliferation and survival in Hodgkin and anaplastic large cell lymphoma. *Blood* 2002, **99**(9), 3398–3403.
64. Jundt F, Probsting KS, Anagnostopoulos I, *et al*. Jagged1-induced Notch signaling drives proliferation of multiple myeloma cells. *Blood* 2004, **103**(9), 3511–3515.
65. Miyamoto Y, Maitra A, Ghosh B, *et al*. Notch mediates TGF alpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell* 2003, **3**(6), 565–576.
66. Apelqvist A, Li H, Sommer L, *et al*. Notch signalling controls pancreatic cell differentiation. *Nature* 1999, **400**(6747), 877–881.
67. Jensen J, Pedersen EE, Galante P, *et al*. Control of endodermal endocrine development by Hes-1. *Nat Genet* 2000, **24**(1), 36–44.
68. Jensen J, Heller RS, Funder-Nielsen T, *et al*. Independent development of pancreatic alpha- and beta-cells from neurogenin3-expressing precursors: a role for the notch pathway in repression of premature differentiation. *Diabetes* 2000, **49**(2), 163–176.
69. Hruban RH, Wilentz RE, Goggins M, *et al*. Pathology of incipient pancreatic cancer. *Ann Oncol* 1999, **10**(Suppl 4), 9–11.
70. Fitzgerald K, Harrington A, Leder P. Ras pathway signals are required for notch-mediated oncogenesis. *Oncogene* 2000, **19**(37), 4191–4198.

71. Ruiz-Hidalgo MJ, Garces C, Laborda J. Notch-1 expression levels in 3T3-L1 cells influence ras signaling and transformation by oncogenic ras. *Int J Oncol* 1999, **14**(4), 777–783.
72. Hasson P, Egoz N, Winkler C, *et al*. EGFR signaling attenuates Groucho-dependent repression to antagonize Notch transcriptional output. *Nat Genet* 2005, **37**(1), 101–105.
73. Berset T, Hoier EF, Battu G, *et al*. Notch inhibition of RAS signaling through MAP kinase phosphatase LIP-1 during C. elegans vulval development. *Science* 2001, **291**(5506), 1055–1058.
74. Capobianco AJ, Zagouras P, Blaumueller CM, *et al*. Neoplastic transformation by truncated alleles of human NOTCH1/TAN1 and NOTCH2. *Mol Cell Biol* 1997, **17**(11), 6265–6273.
75. Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. *Nat Rev Cancer* 2003, **3**(3), 203–216.
76. Grynfeld A, Pahlman S, Axelson H. Induced neuroblastoma cell differentiation, associated with transient HES-1 activity and reduced HASH-1 expression, is inhibited by Notch1. *Int J Cancer* 2000, **88**(3), 401–410.
77. Persson P, Jogi A, Grynfeld A, *et al*. HASH-1 and E2-2 are expressed in human neuroblastoma cells and form a functional complex. *Biochem Biophys Res Commun* 2000, **274**(1), 22–31.
78. Jogi A, Persson P, Grynfeld A, *et al*. Modulation of basic helix-loop-helix transcription complex formation by Id proteins during neuronal differentiation. *J Biol Chem* 2002, **277**(11), 9118–9126.
79. Jogi A, Ora I, Nilsson H, *et al*. Hypoxia alters gene expression in human neuroblastoma cells toward an immature and neural crest-like phenotype. *Proc Natl Acad Sci USA* 2002, **99**(10), 7021–7026.
80. Jogi A, Vallon-Christersson J, Holmquist L, *et al*. Human neuroblastoma cells exposed to hypoxia: induction of genes associated with growth, survival, and aggressive behavior. *Exp Cell Res* 2004, **295**(2), 469–487.
81. Van Limpt VA, Chan AJ, Van Sluis PG, *et al*. High delta-like 1 expression in a subset of neuroblastoma cell lines corresponds to a differentiated chromaffin cell type. *Int J Cancer* 2003, **105**(1), 61–69.
82. Wei JS, Greer BT, Westermann F, *et al*. Prediction of clinical outcome using gene expression profiling and artificial neural networks for patients with neuroblastoma. *Cancer Res* 2004, **64**(19), 6883–6891.
83. Rangarajan A, Talora C, Okuyama R, *et al*. Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. *Embo J* 2001, **20**(13), 3427–3436.
84. Nicolas M, Wolfer A, Raj K, *et al*. Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet* 2003, **33**(3), 416–421.
85. Ito T, Udaka N, Yazawa T, *et al*. Basic helix-loop-helix transcription factors regulate the neuroendocrine differentiation of fetal mouse pulmonary epithelium. *Development* 2000, **127**(18), 3913–3921.
86. Chen H, Thiagalingam A, Chopra H, *et al*. Conservation of the Drosophila lateral inhibition pathway in human lung cancer: a hairy-related protein (HES-1) directly represses achaete-scute homolog-1 expression. *Proc Natl Acad Sci USA* 1997, **94**(10), 5355–5360.
87. Ball DW, Azzoli CG, Baylin SB, *et al*. Identification of a human achaete-scute homolog highly expressed in neuroendocrine tumors. *Proc Natl Acad Sci USA* 1993, **90**(12), 5648–5652.
88. Sriuranpong V, Borges MW, Ravi RK, *et al*. Notch signaling induces cell cycle arrest in small cell lung cancer cells. *Cancer Res* 2001, **61**(7), 3200–3205.
89. Garces C, Ruiz-Hidalgo MJ, de Mora JF, *et al*. Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis. *J Biol Chem* 1997, **272**(47), 29729–29734.
90. Petit A, Bihel F, Alves da Costa C, *et al*. New protease inhibitors prevent γ-secretase-mediated production of Abeta40/42 without affecting Notch cleavage. *Nat Cell Biol* 2001, **3**(5), 507–511.