Notch and cancer: Best to avoid the ups and downs

The Notch pathway is known for its multiple important roles in development and tissue homeostasis, and it can be subverted during oncogenic transformation. Two recent studies add to our understanding of Notch in cancer biology in contrasting ways, by showing novel mechanisms of Notch pathway activation in cancer cells, and by indicating that in some circumstances, Notch can behave as a tumor suppressor.

More than ten years have elapsed since the identification of a balanced translocation involving the human Notch1 gene and the TCRβ locus in a subset of acute lymphoblastic leukemia (Ellisen et al., 1991). This rearrangement leads to expression of a constitutively active truncated Notch allele that behaves as a potent oncogene (for review, see Aster and Pear, 2001). Since this discovery, dysregulated Notch signaling has been hypothesized to be involved in the pathogenesis of a wide range of human neoplasms (for review, see Allenspach et al., 2002). Elucidating the role of Notch in transformation has been greatly enhanced by a markedly improved understanding of the biochemical basis of Notch signaling and its role in normal development. Two reports published in *Nature Genetics* shed new light on Notch and transformation. With the description of a translocation involving a member of the Mastermind-like gene family, an important Notch transcriptional coactivator, in a subset of salivary gland carcinomas, Tonon et al. (2003) unravel novel ways by which the Notch pathway can function as a dominant oncogene. In contrast, Nicolas et al. (2003) show that Notch acts as a tumor suppressor in skin carcinogenesis, where it displays significant interactions with the Wnt and Hedgehog pathways.

These apparently opposite mechanisms by which Notch contributes to cancer should not be a surprise, given that the Notch pathway is used repeatedly but with versatility in multiple processes involving cell differentiation, proliferation, and survival. Although the central components of the Notch pathway are stereotyped, the consequences of Notch signaling are largely dependent on cellular context. The four mammalian Notch receptors (Notch1–4) are transmembrane proteins containing well-defined structural motifs. Activation occurs upon binding of the Notch extracellular domain by a ligand from either the Delta or Jagged families (Figure 1A). After ligand binding, the intracellular domain of Notch (ICN) is released following metalloprotease and γ-secretase mediated cleavages. Signal transduction relies on nuclear translocation of ICN, where it binds CSL, a transcription factor mediating most of the well-characterized Notch functions.

ICN and CSL are part of a large multiprotein complex that contains Mastermind-like (MAML) proteins and other unidentified partners (Wu et al., 2000; Jeffries et al., 2002). Three MAML family members have been identified in mammals (Wu et al., 2002). All three are nuclear proteins with a N-terminal basic domain that binds ICN, and a C-terminal transcriptional activation domain. The respective roles of MAML1-3 are unknown, although their tissue distribution is not overlapping. MAML proteins build a trimolecular complex with ICN and CSL and behave as critical transcriptional coactivators. In vitro data using chromatin-reconstituted templates have shown that MAML1 is essential for Notch-mediated transcriptional activation, perhaps by recruiting p300 and other proteins (Fryer et al., 2002). Assembly of the Notch enhanceosome results in transcription of target genes of the Hairy/Enhancer of Split (HES) and Hairy-related (HRT) families. Additional putative targets include Deltex, $p21^{WAF1\text{-}CIP1}$, LIP1, and preTCRα; however, most transcriptional targets await identification.

The Notch pathway plays a central role in many developmental processes, often involving binary cell fate decisions. Classic examples include peripheral neurogenesis in *Drosophila*, vulval development in *C. elegans*, and lymphoid development in mammals (reviewed in Allman et al., 2002). The normal functions of Notch in T lymphocyte development are likely subverted by oncogenic Notch signaling that causes a differentiation block. Oncogenic Notch probably has additional activities, as inhibition of Notch signaling in Notch-induced T cell leukemia cell lines induces cell cycle arrest and apoptosis (Weng et al., 2003). Similar phenomena occur through retroviral insertional mutagenesis of the Notch4 gene in murine mammary tumors (reviewed in Allenspach et al., 2002).

In their recent paper, Tonon et al. (2003) characterize the t(11;19) translocation found in mucoepidermoid carcinoma, the most frequent subset of salivary gland carcinoma. Inspection of the translocation breakpoint led to the identification of a fusion transcript derived from the first exon of MECT1, a gene of unknown function, and from exons 2–5 of MAML2, a gene with similarity to the previously identified MAML1 (Wu et al., 2000). Involvement of the MECT1-MAML2 protein in the Notch pathway was first suggested by its colocalization with ICN in nuclear speckles of transfected cells. Biochemical studies showed that MECT1-MAML2 binds ICN, although more weakly that full-length MAML2, presumably because MECT1-MAML2 lacks a Notch binding domain encoded by exon 1 of MAML2 (Wu et al., 2002). A role for the fusion protein in mediating oncogenic transformation through activation of the Notch pathway is supported by two sets of data. First, several Notch transcriptional targets such as HRY, HERP1, and HERP2 are upregulated in a mucoepidermoid carcinoma cell line, and can be induced by transfection of MECT1-MAML2 into immortalized parotid ductal cells. Second, MECT1-MAML2 can cooperate with E1A in the transformation of baby rat kidney cells. Since this assay has been used to assess the transforming activity of ICN molecules (Capobianco et al., 1997), these data suggest that activation of the Notch pathway may directly contribute to the oncogenic potential of MECT1-MAML2. Additional studies in transgenic animals will be informative to further characterize the fusion protein. One of the most provocative findings of the study, however, is the unusual way by which MECT1-MAML2 seems to activate Notch target genes in reporter assays. Indeed, activation of the prototypic HES1 promoter by MECT1-MAML2 occurred independently of Notch, and even in the presence of mutated CSL binding sites, suggesting that MECT1-MAML2 can bypass both Notch and CSL to activate

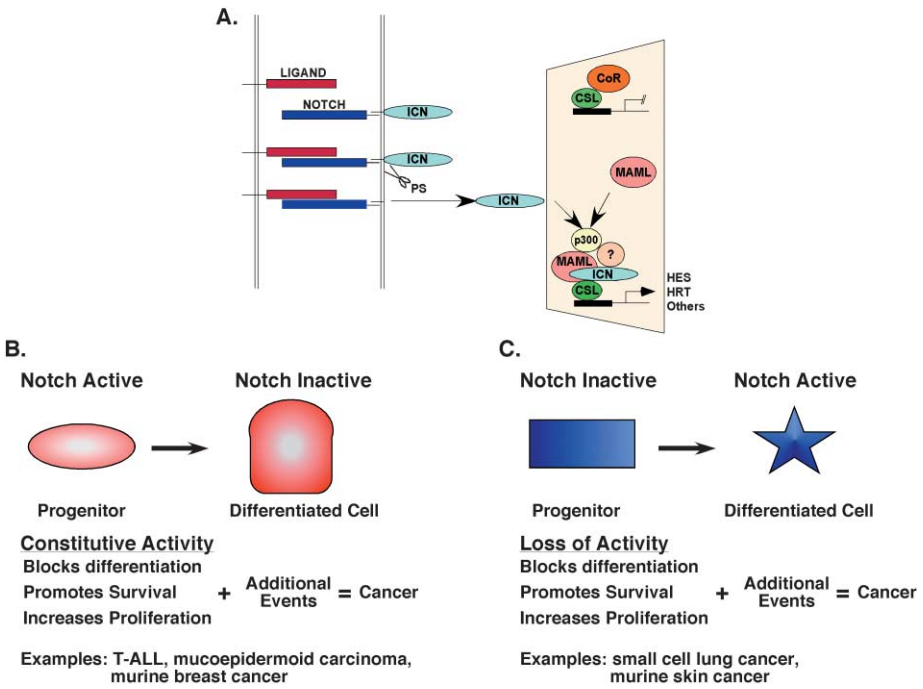

Figure 1. Mechanisms of normal and abnormal Notch signaling

A: Each Notch receptor is expressed as heterodimeric type I transmembrane proteins. Binding of a Notch ligand of the Jagged or Delta-like families triggers a γ-secretase-dependent cleavage of Notch involving Presenilin (PS), which releases the Notch intracellular domain (ICN). ICN migrates to the nucleus, where it forms a large multiprotein complex containing the DNA binding transcription factor CSL, Mastermind-like proteins (MAML), and other proteins. MAML proteins seem critical in mediating Notch-induced transcriptional activation, probably by recruiting p300 and other factors into the transcriptional complex. Target genes include members of the Hairy/Enhancer of Split (HES) and Hairy-related (HRT) gene families.

B: Oncogenic activity of Notch. Schematic representation of the role of Notch in oncogenic transformation, when constitutively expressed in a permissive cellular context.

C: Tumor suppressor activity of Notch. Schematic representation of the putative consequences of Notch loss of function in tissues where Notch physiologically promotes differentiation, such as the skin.

Notch target genes, an unprecedented observation. This hypothesis is reinforced by the observation that MECT1-MAML2 is active in the presence of γ-secretase inhibitors, chemical compounds that block Notch cleavage and nuclear translocation. Studies with $CSL^{-/-}$ cells may help to assess whether the presence of CSL is also nonessential. Understanding how MECT1-MAML2 activates its target promoters and the spectrum of these targets requires additional studies. It will be equally important to understand the contribution of MECT1, since truncated mutants of MAML2 are inactive in the above transcriptional assays. Ultimately, this will provide more specific information about mucoepidermoid carcinoma, as well as new insights into normal and oncogenic Notch signaling.

MAML1 was originally identified through its binding to HPV-E6 (Wu et al., 2000), suggesting that disruption of Notch signaling may play a role in certain cancers. Increasing evidence is accumulating to support this hypothesis. For example, Notch downregulation seems to be critical during tumor progression in small cell lung cancer and in HPV-related cervical cancer (reviewed in Allenspach et al., 2002; Talora et al., 2002). Radtke and collaborators now show that Notch is a tumor suppressor in the skin. Using ICN1 overexpression in primary keratinocytes and Notch1 conditional inactivation in basal epidermal layers, the authors have reported previously that Notch1 promotes differentiation and inhibits keratinocyte proliferation (Rangarajan et al., 2001). In the current work, they show that conditional inactivation of Notch1 in basal epidermal layers leads to spontaneous skin cancer with a basal cell carcinoma (BCC)-like histology. In addition, when exposed to chemical carcinogens, the mice display an increased incidence of tumors with various histological subtypes. Finally, $Notch1^{-/-}$ keratinocytes exhibit an increased sensitivity to Ras-mediated transformation, unlike a previous report in a breast cancer model where Ras-directed transformation was dependent on Notch signaling (Weijzen et al., 2002).

Although these data suggest that Notch1 behaves as a tumor suppressor in the skin, how it exerts this activity remains speculative. Nicolas et al. (2003) suggest several interesting possibilities. The previously identified Notch1-mediated upregulation of $p21^{WAF1-CIP1}$ (Rangarajan et al., 2001) may explain some of the findings, but cannot account alone for the spontaneous tumors, as these have not been observed in $p21^{WAF1-CIP1}$ knockout mice. Interestingly, the authors describe a significant upregulation of downstream mediators of sonic hedgehog (Shh), in particular Gli2, in the skin of induced $Notch1^{-/-}$ animals, and in their keratinocytes during differentiation cultures. Clinical and experimental data indicate that Shh pathway activation is involved in the pathogenesis of BCCs (reviewed in Bale, 2002). Mutations of Patched (Ptc), a negative regulator of the pathway, have been reported in patients with the basal cell nevus or Gorlin syndrome, who have a high incidence of BCCs, while somatic mutations of Ptc occur in sporadic BCCs. Transgenic mice overexpressing Shh, Gli1, or Gli2 develop BCCs. This suggests that the increased Gli2 levels observed in induced $Notch1^{-/-}$ mice contribute to spontaneous tumorigenesis. Genetic interactions between the Notch and the Hedgehog pathways have been described in *Drosophila* wing development (Glise et al., 2002). Whether similar interactions occur in the mammalian skin, and if they are direct or indirect, requires further studies.

Another provocative finding is the upregulation of the Wnt signaling pathway in induced $Notch1^{-/-}$ mice. Genetic data in *Drosophila* show that the two pathways interact, although the molecular mechanisms remain speculative (Axelrod et al., 1996; Glise et al., 2002; Strutt et al., 2002). For example, during *Drosophila* eye development, Notch activity plays a critical role in the control of photoreceptor polarity, in cooperation

with Frizzled, Dishevelled, and other planar polarity genes, possibly through asymmetric localization of these proteins and direct interaction with Notch (Strutt et al., 2002). Similar interactions between Notch and the Wnt pathway may occur in the skin. However, if and how increased Wnt signaling contributes to skin tumorigenesis remains unclear. Both in humans and mice, activating mutations of β-catenin have been linked to pilomatricomas, a tumor of hair follicles, but not to other types of skin cancer (Chan et al., 1999). Although enhanced β-catenin signaling has been tentatively linked to skin tumorigenesis in Presenilin1 (PS1) knockout mice (Xia et al., 2001), PS1 deficiency may result both in increased β-catenin stability and defective Notch processing, which could contribute to the skin tumors.

In summary, these studies illustrate the versatile effects of Notch in cancer (Figures 1B–1C). The precise oncogenic effects of either loss or gain of Notch function on development, proliferation, and survival remain to be elucidated. Nevertheless, the current studies further emphasize that the consequences of aberrant Notch signaling depend on cell context, dose, and timing. In addition, these findings identify new obstacles in manipulating the Notch pathway for therapeutic purposes, while at the same time expanding the targets where this type of therapy may be useful.

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