Lasting longer without oxygen: The influence of hypoxia on Notch signaling

Notch signaling has multiple functions during invertebrate and vertebrate development, where it plays important roles in cell fate determination, proliferation, survival, and border formation. The precise function of Notch signaling is context dependent, and one of the unsolved mysteries of Notch signaling is how a relatively stereotyped signal transduction pathway exerts such a wide variety of context-specific responses. Recent data from Gustaffson et al. provide important new information on this topic by showing that hypoxia enhances Notch signaling due to the association of Notch and HIF-1α. This interaction may have important consequences for tumor cell growth.

Low levels of tissue oxygen, or hypoxia, can be caused by coronary artery disease, stroke, or the growth of solid tumors. Hypoxic microenvironments also occur during embryogenesis and in the adult, where one consequence may be the creation of niches that maintain pluripotential cells (Simon et al., 2002). Work in hematopoietic and neural stem cells showed that culturing progenitors in hypoxic conditions (3%–5% O_2) increased the number of multipotent clones in comparison to normoxic cultures (21% O_2) (Adelman et al., 1999; Morrison et al., 2000). In addition, culturing progenitors under hypoxic conditions leads to different developmental outcomes than culture in normoxic conditions. For example, culturing CNS precursors under hypoxic conditions skews differentiation toward the dopaminergic fate. Besides effects on differentiation and cell fate, hypoxia promotes survival and increases the proliferation of multipotent precursors.

Some of the effects of hypoxia on stem cells correlate with the effects of Notch signaling on these cells. Notch is able to both maintain the pluripotential state of some cells and induce specific cell fates. Notch also influences proliferation and survival. In the November issue of *Developmental Cell*, the studies of Gustaffson et al. provide important evidence showing that hypoxia directly influences Notch signaling (Gustafsson et al., 2005). In this work, hypoxia caused HIF-1α to potentiate Notch activation. In both myogenic and neural precursor cell lines, hypoxia inhibited differentiation, an effect that was abrogated in the presence of γ-secretase inhibitors, which can inhibit Notch signaling. In P19 embryonic carcinoma cells, which undergo neuronal differentiation in response to Notch signaling, hypoxia caused a 4-fold increase in the Notch transcriptional target Hes1. The hypoxic effects were mimicked by known promoters of HIF-1α stability, suggesting that HIF-1α directly mediates the effects of hypoxia on Notch. The association between HIF-1α and Notch was confirmed by immunoprecipitation of in vitro translated proteins, which identified both proteins in the same complex. Although the Notch:HIF-1α association did not require the HIF-1α C terminus, which is required for p300 recruitment, enhanced transcriptional activation via Notch did require the HIF-1α C terminus and presumably p300 recruitment.

It is of particular interest that hypoxia appeared to enhance the stability of intracellular Notch (ICN). In P19 cells, hypoxia caused a HIF-1α-dependent increase in ICN levels, suggesting that HIF-1α stabilizes ICN. Similar results were obtained in HIF-1α-transfected COS7 cells. Normally, nuclear ICN is a very short-lived protein. Mammalian Notch signaling is initiated when ligands of either the Delta or Jagged family bind one of four Notch receptors (Figure 1A). This results in a series of proteolytic cleavages, the first mediated by an ADAM metalloprotease and the second by γ-secretase. The final cleavage releases ICN (Selkoe and Kopan, 2003), which contains a set of functionally crucial ankyrin repeats, a C-terminal PEST domain, and two nuclear localization sequences that guide ICN into the nucleus. Once there, ICN recruits a member of the Mastermind family (MAML) and other transcriptional activators, such as p300. Recent work has shown that transcriptional activation by Notch requires the ternary transcriptional activation complex of CSL, ICN, and MAML (Fryer et al., 2002; Wallberg et al., 2002). Normally, nuclear ICN is extremely short lived; in early studies, its existence was largely inferred indirectly from reporter gene and functional assays (Mumm and Kopan, 2000). Recent work suggests that MAML functions not only in transcriptional activation (which involves recruitment of p300), but also in ICN turnover (Fryer et al., 2002, 2004; Wallberg et al., 2002). The latter activity is correlated with phosphorylation of the Notch C-terminal PEST domain by CycC:CDK8 (Fryer et al., 2004), an event that may promote PEST-dependent degradation by the Fbw/Sel10 ubiquitin ligase.

Although the data from Gustaffson et al. showed that ICN and HIF-1α can be coimmunoprecipitated, their precise relationship is unclear (Figure 1B). One possibility is that HIF-1α is recruited to the CSL:ICN:MAML ternary complex. In this scenario, HIF-1α could interfere with the ability of MAML to promote the degradation of ICN, and thereby prolong Notch signaling. Another possibility is that HIF-1α replaces MAML in the ternary complex. Data from the Jones and Roeder groups showed that MAML recruits p300 to the complex (Fryer et al., 2002; Wallberg et al., 2002); thus, HIF-1α, whose activity requires p300, would bind Notch and bring p300 into the complex. Again, in this scheme HIF-1α is less efficient than MAML in promoting ICN degradation. Another possibility is that ICN and HIF-1α associate indirectly in the same complex through contacts with other proteins. Although p300 is an attractive candidate, the structure/function studies of Gustaffson et al. appear to rule this out, as HIF-1α mutants that are unable to bind p300 coimmunoprecipitated with ICN. Other questions raised by the Gustafsson et al. findings are whether HIF-1β associates with the complex and how to interpret the association of the HIF-1α inhibitor FIH with ICN.

The ICN:HIF-1α complex appears to stimulate CSL-dependent Notch signaling. This raises the question of whether hypoxia regulates transcription of all CSL:Notch transcriptional targets or only a subset. In the current work, only two promoters were examined, Hey-2 and Hes-1, both of which are activated by Notch in a wide variety of cell types and contexts. Whether hypoxia selects for a specific subset of Notch targets remains to be determined. It is also not

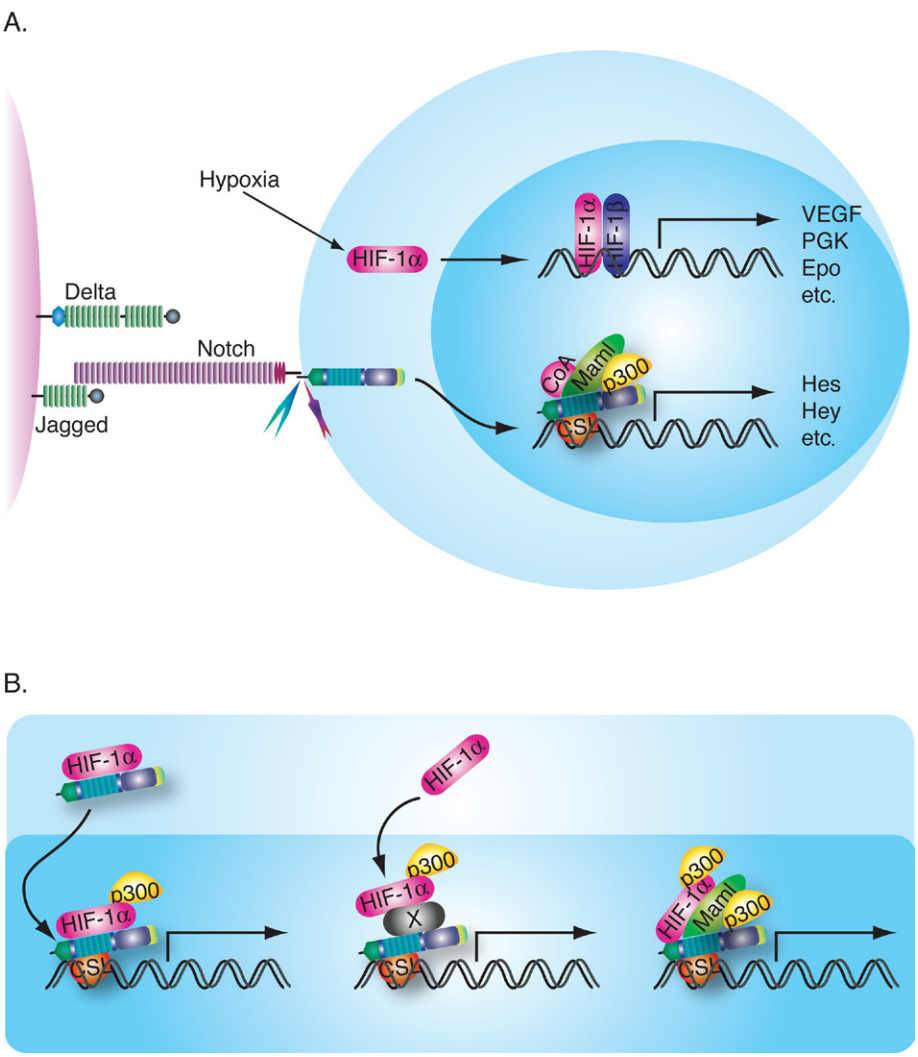

Figure 1. Models depicting potential interactions of HIF-1α with ICN

A: The canonical HIF-1α and Notch:CSL signaling pathways are shown. Hypoxia ultimately leads to creation of the HIF-1α:HIF-1β transcriptional activation complex that initiates transcription of specific genes, some of which are shown. The details of the Notch signaling pathway are discussed in the text.

B: Potential interactions of HIF-1α with ICN in the nucleus. The left panel shows a direct interaction of HIF-1α with ICN. In this model, HIF-1α substitutes for MAML. It is not known if the HIF-1α interaction first occurs outside or within the nucleus. In the middle panel, HIF-1α interacts with ICN through a bridging protein. One possibility is MAML. As discussed in the text, p300 is unlikely. In the right panel, both MAML and HIF-1α are recruited to the CSL:ICN transcriptional activation complex. Note that both HIF-1α and MAML can bind p300.

known whether HIF-1α interacts with all four mammalian Notch receptors. The experiments of Gustafsson et al. relied on overexpression of ICN1 or chromatin immunoprecipitation with Notch1-specific reagents, leaving open the question of whether similar effects are exerted by the other Notch receptors.

In addition to HIF-1α, other proteins have also been shown to modulate ICN turnover in both normal and pathological situations. Artavanis-Tsakonis and colleagues recently identified a trimolecular complex between Notch, Deltex, and the nonvisual β-arrestin Krz, which enhanced degradation of ICN (Mukherjee et al., 2005). Recent work from the Aster and Look labs showed that the majority of human T cell lymphoblastic leukemias (T-ALL) have Notch1 mutations (Weng et al., 2004). At least one-third of these tumors have mutations that create frameshifts or introduce premature stop codons that result in deletion of the C-terminal PEST sequences and prolonged ICN1 half-life. These data indicate that dysregulated control of nuclear Notch turnover plays a role in certain human cancers.

In contrast to T-ALL, C-terminal Notch mutations have not been identified in other human tumors. Yet many tumors, such as breast cancer and pancreatic cancer, show elevated levels of Notch expression and signaling (Radtke and Raj, 2003). An interesting possibility is that hypoxia, rather than Notch mutations, leads to enhanced Notch signaling in these cells through HIF-1α expression. Whether this is functionally important can be addressed by treating tumor cells with inhibitors of the canonical Notch signaling pathway. Such experiments will not only reveal whether the elevated levels of Notch signaling influence the growth, survival, and/or differentiation of tumor cells, but may provide a rationale for new therapeutic interventions directed at the Notch pathway.

Although the Gustaffson et al. paper focuses on cell-autonomous effects of Notch signaling, hypoxia alters the tumor microenvironment as well. Accumulating evidence shows that Notch plays an important role in establishing arterial fate (Alva and Iruela-Arispe, 2004). Recent work also demonstrated a potential role for Notch in tumor angiogenesis. Zeng et al. reported that MAPK-dependent upregulation of the Notch ligand Jagged1 in some tumor cells promoted Notch-dependent tumor vascularization (Zeng et al., 2005). Whether HIF-1α influences this function of Notch is not known. An interesting possibility from the Notch:HIF-1α association is that small molecule inhibitors of HIF-1α (Kong et al., 2005) may serve as context-dependent inhibitors of Notch signaling

In summary, the exciting work of Gustafsson et al. provides a new vantage from which to consider the influence of Notch signaling. Although much of the details remain to be determined, there appears to be a close link between hypoxic signaling and Notch. As both of these pathways are associated with multiple aspects of neoplasia, it will be important to determine the precise contexts in which these pathways influence the transformed state. Hopefully, these results will guide the development of therapeutics based on the intersection of Notch and hypoxic signaling.

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A knotty turnabout?: Akt1 as a metastasis suppressor

Akt is well known to enhance malignancy and is recognized as a key target for antineoplastic therapies. However, intriguing findings reported by Yoeli-Lerner et al. in the November 23, 2005 issue of *Molecular Cell*, suggest a novel, antimetastasis function of Akt: activation of Akt1 inhibited invasion in some cancer cells. One possible mechanism for this surprising phenotype was that Akt activated the E3 ubiquitin ligase HDM2, causing ubiquitination and degradation of NFAT, an invasion-promoting factor. These findings clearly justify further investigations and, if validated in vivo, call for reevaluation of some Akt-targeting therapeutic strategies currently under development.

Akt modulates a variety of cellular processes, including proliferation, growth, and survival, by phosphorylating target proteins involved in these processes (recently reviewed in Bellacosa et al., 2004; Brazil et al., 2004; Osaki et al., 2004; Woodgett, 2005). Many signaling pathways activate Akt through phosphatidylinositol-3-kinase (PI3K). PI3K converts phosphatidylinositol-4,5-bis-phosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3). PIP3 recruits Akt to the membrane where Akt is activated. PTEN dephosphorylates PIP3 and antagonizes PI3K function. Additionally, several molecules, such as CTMP, TRB3, and PHLPP, bind to and directly regulate Akt (Brazil et al., 2004; Gao et al., 2005).

Well-characterized substrates of Akt include antiapoptotic proteins, such as FOXO, BAD, and IKK-β; cell cycle regulators, such as $p27^{kip1}$, $p21^{cip1}$, MDM2, and Myt1; and GSK-3, which is involved in a variety of processes (Brazil et al., 2004). Akt has also been shown to stimulate angiogenesis via regulation of eNOS and increase cell metabolism through the mTOR/p70S6K pathway (Luo et al., 2003). Additionally, Akt stimulates MMP secretion, activates the small GTPase Rac, and promotes epithelial-to- mesenchymal transition (EMT), three attributes that can lead to increased metastatic potential (Bellacosa et al., 2004; Luo et al., 2003; Zhou et al., 2004). In light of Akt's involvement in all these pathways, it is not surprising that Akt protein levels, enzymatic activities, and even gene copy numbers are increased in many different types of tumors. Akt upregulation has been seen in carcinomas of the prostate, breast, ovary, pancreas, colon, stomach, and thyroid (Luo et al., 2003; Osaki et al., 2004). The importance of increased Akt activity in tumorigenesis is further underscored by the identification of gain-of-function mutations in PI3K and loss of expression of the PTEN tumor suppressor gene in many tumors, which positively and negatively regulate Akt activation, respectively (Luo et al., 2003; Osaki et al., 2004; Saal et al., 2005).

Given these well-established mechanisms by which Akt activation promotes transformation, a publication by the Toker laboratory in the November 23, 2005 issue of *Molecular Cell* suggests a surprising twist on Akt's role in the tumorigenic process (Yoeli-Lerner et al., 2005). Compelling in vitro evidence indicates that Akt blocked motility and invasion, important metastasis-related properties, in three different breast cancer cell lines. This was studied by artificially activating Akt1 and also by activating Akt with IGF-1 as a physiological stimulus. Conversely, lowering Akt1 levels with siRNA restored, and in some cases enhanced, the invasive properties of the cells. The kinase activity of Akt was