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Tuning NOTCH

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Introduction

The emergence of multi-cellular organs and their organization into complex body plans is a major cornerstone concerning origin of life on Earth. This requires that the fundamental unit of life, namely the cell, must communicate, coordinate and organize. Cell communication mediated by signal transduction pathways is essential for the development and functioning of any organism. About 20 different signal transduction pathways are required to generate the high diversity of cell types, patterns and tissues characteristic of metazoans [1]. A major juxtacrine signaling system that allows cells to directly talk to each other to program almost every cell type in the body is characterized by the Notch pathway.

The observation of Notched-wings in the fruit fly *Drosophila melanogaster* in the year 1914 by John Dexter, and subsequent attribution of this phenotype to Notch gene in 1917 by Thomas Morgan [2] led to the discovery of the exquisitely complex Notch pathway that represents one of the major communication channels between neighboring cells [3,4]. Notch signaling is heavily dependent on contextual cues such as physical interactions with the tissue microenvironment and cross-talk with other signaling pathways, without requiring any secondary messenger system.

A genome-wide comparative study has revealed 22 main components of the evolutionarily ancient Notch pathway [5]. While many of the Notch pathway components are shared with non-metazoan eukaryotes, nine of the components encoding canonical Notch receptors and ligands are metazoan-specific. This indicates that the Notch pathway has evolved through the co-option of ancient pre-metazoan proteins, and their integration with novel metazoan-specific molecules. This pathway has remained highly conserved throughout evolution. Although some components of the Notch pathway were present in fungi, amoeba, and plants as well as in the earliest excavata, a functional Notch pathway emerged in the last common ancestor of present-day metazoans, the Urmetazoa [5].

There are inter-species differences between the precise numbers of Notch paralogues, for example, mammals have four Notch receptors (Notch 1-4), *Caenorhabditis elegans* has two (LIN-12 and GLP-1) and *Drosophila melanogaster* has one (Notch), but the basic signaling framework is common throughout [6,7]. Notch has two families of ligands encoded by the two paralogous genes *Delta* and *Jagged*. *Delta* was ancestrally present in Metazoa, whereas a complete Jagged is absent in Placozoa and Porifera. Both the Notch receptor and its ligands, *Delta* and *Jagged* (Serrate in *Drosophila*) are transmembrane proteins with large extracellular domains that consist primarily of Epidermal Growth Factor (EGF)-like repeats. There are five mammalian Notch ligands Delta-like 1, 3 and 4 (DLL1/3/4) and Jagged 1 and 2 (JAG1/2) [7]. The Delta ligands trans-activate Notch in neighboring cells and *cis*-inhibit Notch in its own cells [8,9].

Notch signaling is unique from other conserved signaling pathways in its mechanism of signal transduction. It relies on the ability of a ligand to bring about receptor proteolysis, resulting in the release of an active Notch fragment. A second unusual feature is that intra-membrane proteolysis is involved in its receptor activation. After its proteolytic release from an intra-membranous tether, the Notch Intracellular Domain (NICD) translocates to the nucleus. There, it associates with a DNA-binding protein to assemble a transcription complex that activates downstream target genes. This forms the core signal transduction pathway in most "canonical" Notch signaling processes [4]. Since each Notch receptor molecule undergoes proteolysis to generate a signal and thus can only signal once, the availability of either Notch ligand or receptor at the cell surface is key to controlling Notch activation.

The "noncanonical" Notch signaling involves three possible scenarios: (1) transduction of activation signals following Notch ligation and nuclear translocation of NICD independent of hetero dimerization with the DNA-binding transcription factor CSL (named after CBF1 for human, Suppressor of hairless Su(H) for *Drosophila*, and LAG-1 for *C. elegans*; also known as RBP-J in mouse); (2) activation of Notch target genes independent of γ-secretase-mediated NICD cleavage and CBF1; and (3) CBF1-dependent activation without ADAM family metalloprotease-mediated Notch receptor cleavage and γ-secretase-mediated NICD cleavage. Such noncanonical Notch pathways most likely represent a point of cross-talk between other classical intracellular signaling pathways, including Hedgehog, Jak/STAT, RTK, TGF, Wnt, PI3, mTor, Akt, JNK, MEK/ERK, and NFκB [4,10-12].

The Notch signaling with promiscuous receptor-ligand binding is highly dose- and context-dependent. It regulates numerous critical cell fate specification events during the ontogeny of the nervous system, hematopoietic system, eye, and skin via the developmental processes of lateral inhibition and boundary induction [4,7]. In addition, it also plays diverse roles in regulating malignant hematopoiesis, maintenance and expansion of lineage-restricted hematopoietic progenitors, lymphocyte differentiation, and peripheral immune responses. Evidence is also accumulating to indicate that the deregulation of Notch plays important role in the development of both normal and cancer stem cells [13]. Moreover, Notch – like all oncoproteins – can contribute to the process of tumorigenesis as long as it partners with another onco-signaling. For example, crosstalk between Notch and TGFβ is important for the Epithelial-Mesenchymal Transition (EMT) as Notch signaling is required to sustain TGFβ-induced Notch target gene *hey1*

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both tumour-promoting and -suppressive roles within the same tissue [15].

Besides the established roles of the Notch system described above, our recent discovery of its significance for antitumor effector functions and memory [16] reveals important gaps in current knowledge. Since the Notch system lies at a juncture of an interactive cell signaling network, activation or inhibition of selective Notch markers may serve as unique predictors of immune reconstitution, re-established tumor immunosurveillance and direct anticancer effects following cancer immunotherapy. Our work revealed that tumor and its derivative pro-angiogenic factors such as the Vascular Endothelial Growth Factor (VEGF) [17,18] down-regulates the expression of Delta-like Notch ligands in the hematopoietic compartment of mice as well as humans, and the resulting decrease in ligand-induced Notch activation leads to defects in T cells [16]. Paradoxically, we also recently discovered that stimulation of DLL1-mediated Notch signaling by over-expression of DLL1 in the hematopoietic compartment or by systemic administration of a prototypic therapeutic reagent, clustered multivalent DLL1, was sufficient to correct tumor-induced defects in T lymphocyte differentiation, and enhance T cell anti-tumor immunity to produce significant tumor inhibition in mouse lung cancer models [16]. Thus, modulation of DLL1/Notch signaling has the exquisite potential to enhance antitumor T cell immunity by overcoming tumor-associated immunosuppression.

Depending upon the cellular context and the levels of Notch-regulatory factors in cells, the amplitude and duration of Notch activity is amenable to regulation at various points in the pathway. In some contexts, continuous Notch signaling is required; in others a transient Notch signal is sufficient to influence cell fate and differentiation. Notch along with two downstream transcription factors Hes1 and RBP-J forms an intricate network of positive and negative feedback loops. The Notch system exhibits a bistable activity and is capable of switching states at a critical level of Notch signaling initiated by its ligand Delta. As Hes1 levels can change as a function of the input Delta signal, a transient pulse of a high level of Delta is capable of inducing high expression of *Hes1* for a duration that would be sufficient to induce a binary cell fate switch. For example, transient Delta-Notch signaling has been shown to be sufficient to induce T cell [19] or NK cell differentiation [20]. The prolonged expression of *Hes1* upon transient Delta activation is due to the long half-life of NICD [21]. The bistable switch is thus sensitive to the degradation of NICD. Integrin-Linked Kinase (ILK), which is either activated or overexpressed in many types of cancer including breast cancer [22], can reduce the protein stability of Notch 1 and thus decrease NICD half-life. High ILK and low NICD levels are detected in basal cell carcinoma and melanoma patients [23].

The Notch can transition from functioning as a bistable switch to an oscillator by tuning the transcriptional repressor gene *hes1*. The expression levels of members of the *hes* family have been shown to oscillate with a 2 hour periodicity [24,25]. Such oscillations may afford cells the opportunity to test for the continued existence of a signal. This may attune the cellular response by allowing the cell to integrate the results of many periodic evaluations of the signal in conjunction with other signal transduction cues before delivering a final outcome. Thus, cells can fine-tune the context-dependent non-linear Notch signaling in different cellular settings presenting a highly interconnected cell communication network to produce a variety of downstream cell fates and functions. Further investigations into the mechanisms on how modulation of Notch circuitry can deliver diverse outcomes in different contexts are valuable not only from a basic biology standpoint but also from therapeutic medicine viewpoint.

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