Nuclear receptor regulation gears up another Notch

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In this perspective we describe examples of crosstalk between nuclear receptors (NRs) and Notch signaling by means of direct functional interactions between components of both pathways. This crosstalk may provide eukaryotic organisms with molecular mechanisms for the coordination of llong-distance endocrine signals with cell-to-cell juxtacrine communication.

Received September 13th, 2005; Accepted Novemeber 15th, 2005; Published February 8th, 2006 | **Abbreviations: AR:** androgen receptor; **bHLH-PAS:** basic-helix-loop-helix/Per-Arnt-Sim; **BPH:** benign prostatic hyperplasia; **HDAC:** histone deacetylase; **HES:** hairy and enhancer of split; **HEY1:** Hairy/Enhancer of split related with YRPW motif 1; **NR:** nuclear receptor; **SRC1:** steroid receptor coactivator 1

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Introduction

The ability of nuclear receptors (NRs) to regulate transcription from target genes depends on the recruitment of chromatin remodeling enzymes and the transcription machinery. More than 100 different NR co-regulators have been reported, many of which may also mediate the function of other transcription factors. The function of these proteins is subject to regulation at several levels, including their rate of expression, protein stability, postranslational modifications, changes in subcellular localization, and allosteric modifications. In addition, cross-coupling between different signaling pathways may play an important role in creating the myriad of responses required in higher eukaryotic organisms. We have identified a novel mechanism of crosstalk between the androgen receptor and Notch signaling pathways with potential implications in the development of prostate cancer. Our results, together with recent reports describing other mechanisms of crosstalk between NRs and Notch, suggest that these two signaling pathways could have unexpected functional interactions.

Characterization of HEY1, a mediator of Notch signaling, as an androgen receptor corepressor

SRC1 is a member of the p160 family of coactivators that play a central role in the regulation of NR transcriptional activity, interacting directly with them and acting as platform for the recruitment of the enzymatic activities responsible for the regulation of gene expression [Glass and Rosenfeld, 2000; Leo and Chen, 2000; McKenna and O'Malley, 2002; Onate et al., 1995]. In an attempt to identify the protein interactions required for the regulation of NR activity we performed a yeast two-hybrid screen with the highly conserved bHLH-PAS N-terminal domain of SRC1, a putative protein interaction region. One of the proteins we found was later characterized as a downstream target of Notch signaling pathways, named HEY1(Hairy/Enhancer of split related with YRPW motif 1) a member of the bHLH-Orange domain superfamily of transcriptional repressors [Davis and Turner, 2001; Iso et al., 2003]. Although it was initially surprising to find an interaction between a well characterized NR coactivator and a transcriptional repressor, genetic and biochemical evidences led us to study possible effects on androgen receptor (AR) transcriptional activity. We found that HEY1 interacts directly with both SRC1 and the AR and demonstrated that it could function as a repressor of AR [Belandia et al., 2005]. None of the other steroid receptors tested was repressed by HEY1 and, to our knowledge, AR is the only NR inhibited by HEY1.

Eukaryotic transcriptional repressors can act in a passive way, by forming inactive heterodimers with transcriptional activators, by sequestering coactivators, or by competing with positive transcription factors for DNA binding. On the other hand, active transcriptional repressors possess intrinsic repressing activities that recruit enzymatic activities such as histone deacetylases (HDACs) and ATP-dependent chromatin remodeling factors, contributing to compact the chromatin fiber. HEY1 can bind to E-box motifs in the DNA and it also contains at least two independent domains with autonomous repressing activity; a HDAC dependent C-terminal region, and the N-terminal bHLH domain that represses through both HDAC-dependent and HDAC-independent mechanisms. The bHLH domain interacts with Sin3/NCoR complexes that mediate the recruitment of HDACs, non-covalent nucleosome remodeling complexes and histone methytransferases. HEY1 mediated repression of the AR requires the concerted action of both repressive domains suggesting that the repression reflects a combination of multiple repressing mechanisms.HEY1, like other HEY and HES genes, is a target gene for Notch signaling. Notch receptors are transmembrane proteins that interact with their ligands present in the surface of adjacent cells. Upon Notch ligand binding, the notch intracellular domain migrates to the nucleus and induces the expression of primary targets of Notch signaling, such

as HEY and HES genes [Iso et al., 2003; Kadesch, 2004]. Increasing HEY1 expression blocks the ability of SRC1 to potentiate AR transcriptional activity, therefore, changes in the expression of endogenous levels of HEY1 in the cell, induced by Notch activation, have the potential to modulate the cellular responses to testosterone.

Is there a reciprocal negative feedback between Notch and AR-dependent pathways in the prostate?

Gene profiling experiments have identified a number of signaling pathways, including notch signaling, that may be subject to regulation by testosterone in the prostate [Nantermet et al., 2003]. Thus NOTCH1 and its ligand JAGGED1 were repressed and a negative regulator of Notch signaling, SEL1L, was induced, indicating that testosterone might inhibit Notch signaling. These results, along with our observed repression of AR by HEY1, a Notch target, provide a direct mechanism for reciprocal negative feedback between androgen-dependent gene regulation and Notch signaling (Figure 1). Accordingly, this crosstalk could contribute to the coordination between 'long-distance' endocrine signaling exhibited by steroid hormones, and cell-to-cell juxtacrine signals transmitted by Notch receptors.

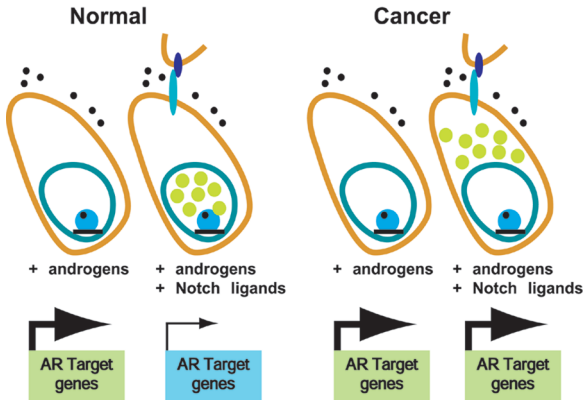

Figure 1. Proposed mechanism for crosstalk between the Androgen Receptor and Notch signaling pathways. The AR and Notch receptors play essential roles in the regulation of prostate development and homeostasis. Notch signaling initiates when receptor-bearing cells interact with Notch ligands present in neighboring cells. Notch activation causes an increase in HEY1 expression and HEY1 accumulates in the nucleus repressing AR transcriptional activity. In a reciprocal way, the activation of AR upon androgen binding downregulates the expression of Notch1 receptor and its ligand Jagged1, and upregulates Sel1L, a negative regulator of Notch. These changes in gene expression presumably will inhibit signal transmission via Notch receptors. This crosstalk can help to modulate both pathways, integrating an endocrine signal with cell-to-cell communication in a coordinate response.

Implications for prostate cancer

Recent reports demonstrated an important role for Notch signaling in the regulation of normal prostate development [Shou et al., 2001; Wang et al., 2004]. In addition, the expression of NOTCH1 and its ligand JAGGED1 is elevated in malignant prostatic epithelial cells and it has been proposed that dysregulation of JAGGED1 plays a role in prostate cancer progression and metastasis [Santagata et al., 2004; Shou et al., 2001]. We compared HEY1 expression between human primary prostate tumors and benign prostatic hyperplasia (BPH) and we found a striking difference in the localization of HEY1 protein in malignant cells. The majority of BPH samples showed strong nuclear HEY1 staining whereas its expression was restricted to the cytoplasmic compartment of prostate carcinoma cells (in 8 out 10 patients). The nuclear exclusion of HEY1 did not affect AR expression, which was localized mainly in epithelial cell nuclei in both BPH and prostate cancer. Our proposed mechanism for repression of AR-target genes via induction of Notch signaling depends on the accumulation of HEY1 in the nucleus, therefore its nuclear exclusion in cancer cells would remove a brake for AR action. Thus, cancer cells would become insensitive to Notch ligands and proliferate despite contact inhibition signals received by Notch receptors (Figure 2). In addition, other metabolic pathways repressed by HEY1 might also be affected.

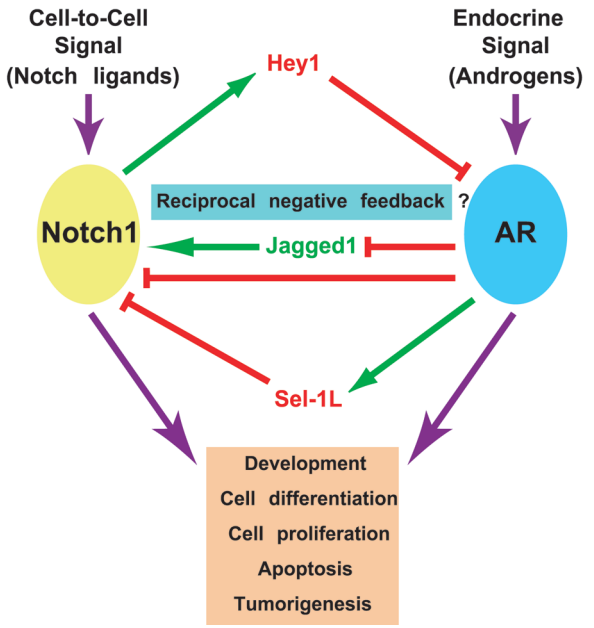

Figure 2. Hypothetical model describing how alterations in HEY1 subcellular localization could contribute to an abnormal response to androgens in prostate cancer cells. In normal cells, upon androgen binding, AR binds to its cognate response elements in the promoter of target genes and induces gene expression. The activation of Notch signals in the same cells leads to the accumulation of the repressor HEY1 in the nuclei, reducing the expression of AR-dependent genes. In cancer cells, if HEY1 is excluded from the nuclei, it cannot repress AR action. Thus, cancer cells are insensitive to AR modulation via Notch signals mediated by HEY1.

We need to analyze more cancer samples before we can generalize our observations, but our findings expand the list of possible causes for the appearance of androgen-independent prostate cancer. It will be of interest to investigate nucleocytoplasmic shuttling in cancer cells and determine whether defective mechanisms arise that might be restricted to certain signaling molecules that could offer a proliferative advantage. The mechanisms that regulate HEY1 subcellular localization are still unknown. Sequence analysis has identified several regions in HEY1 homologous to nuclear export and nuclear localization

consensus signals and mutational studies of these regions should give us clues regarding the mechanisms regulating HEY1 transport within the cell. Prostate cells depend on androgens for growth control and to avoid apoptosis, therefore nearly all advanced androgen-independent prostate tumors retain AR expression. Extensive research has described many mechanisms that contribute to the escape of AR to normal regulation, including mutations in the AR, changes in its expression levels and/or its coactivators or aberrant stimulation through crosstalk with peptide growth factors and cytokines. Our results suggest that it might be a selection for reduced HEY1 dependent Notch signaling in prostate cancer. However, HEY1 is only one of the many molecular components of the complex cascades responsible for transmitting Notch signals. Notch signaling can suppress or promote tumorigenesis depending on the cellular context and the crosstalk with other signal transduction pathways [Radtke and Raj, 2003]. We need first to address the role of Notch signaling in the normal prostate gland before we can understand its role in the initiation and progression of prostate cancer and the functional consequences of crosstalk with AR pathways. To do so, animal models with prostate-specific ablation of different components of the Notch pathway will be invaluable tools. This knowledge should provide new insights into the biology of prostate cancer and the possible involvement of Notch signaling in the communication between AR-positive and AR-negative cells within prostate tumors.Growing evidences for a link between NRs and Notch signaling pathwaysRecent work has implicated Notch signaling in another endocrine cancer, namely hormone responsive breast cancer. Thus, estrogens can increase the expression of JAGGED1 and NOTCH1 in breast cancer cells and this activation seems to be mediated, at least in part, by estrogen-responsive elements present in their promoters. It has been proposed that estrogen-dependent activation of Notch signaling plays a role in the regulation of angiogenic processes [Soares et al., 2004]. In addition, a clinical study has described high level coexpression of JAGGED1 and NOTCH1 in human breast cancer that is associated with poor overall patient survival [Reedijk et al., 2005], reinforcing the possibility that Notch signaling is important for tumor progression in estrogen-dependent tissues.

Conclusion

Crosstalk between NRs and Notch signaling pathways seems to constitute one of the mechanisms by which organisms integrate long distance endocrine signals with cell-to-cell communication between adjacent cells helping to build up the complexity required in higher eukaryotic organisms. We predict that more examples of coordinated action of these two signaling pathways will be uncovered in the near future. The elucidation of the molecular mechanisms responsible for the crosstalk between Notch pathways and NRs will provide insights into many physiological processes that are subject to regulation by endogenous ligands. Pharmacological antagonists are widely used for the treatment of endocrine cancers such as breast and prostate cancer. The examples mentioned above in which Notch signaling and NRs modulate their activities in a reciprocal way with potential implications for the appearance of androgen-independent prostate cancer, the prognosis of estrogen-dependent breast tumors or the regulation of angiogenic processes suggest that the identification of the detailed molecular mechanisms of interference between NR and Notch pathways might help us to develop novel therapeutic strategies for endocrine cancers.

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