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REVIEW

The role of Notch signaling in human cervical cancer: implications for solid tumors

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The detection of intracellular forms of Notch1 in human cervical cancers more than a decade ago prompted an investigation into the possible role of this pathway in driving these cancers. These tumors are consistently characterized by features of deregulated ligand-dependent signaling. Although Notch signaling complements the function of papillomavirus oncogenes in transformation assays of human keratinocytes, there are dose-dependent effects, which inhibit growth of established cervical cancer cell lines. Two pro-oncogenic effector mechanisms that have been suggested to operate in this context by Notch signaling are the activation of PI3K/Akt pathway and the upregulation of c-Myc. Collectively, there is a complex interplay between Notch signaling and papillomaviruses in the context of cervical carcinogenesis. Better animal model systems and identification of human cervical cancer stem cells should help clarify the possible stage specific and pleiotropic effects and regulation of Notch signaling.
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The bulk of human cancers are solid tumors of epithelial origin. The link of Notch signaling to murine solid tumors first emerged from mouse mammary tumor virus (MMTV) integration assays, a classical method used to isolate host genes that markedly influence tumor progression (Robbins *et al*., 1992). Of the four genes that were detected and analysed in the context of murine mammary tumor progression, two turned out to be members of the Wingless gene family. The third and fourth genes characterized in this subset were members of the fibroblast growth factor and Notch gene family (Int-3), respectively (Robbins *et al*., 1992). Artavanis-Tsakonas and co-workers first extended the possible role of Notch signaling to human solid tumors in addition to murine models. They reported that an antibody against the human *Notch1* gene product detected intracellular forms of the protein in human cervical cancers (Zagouras *et al*., 1995). Daniel *et al*. (1997) subsequently reported that there appeared to be a greater detection of these intracellular forms in invasive human cervical cancers as opposed to high-grade precursor lesions.

Cancer of the cervix is one of the common cancers in women in the developing world. There is conclusive evidence of linkage between high-risk human papillomaviruses (HPVs) and cervical cancer (zur Hausen, 2002). Squamous and columnar epithelium cover the ectocervix and the endocervical canal, respectively. At the squamocolumnar junction (transition zone) there are reserve cell populations that can differentiate into squamous or columnar cells. The integration of HPV genomes as well as the initiation of neoplastic transformation is believed to occur at this region (Reid, 1983). The integration results in disruption of the viral E2 open reading frame, a repressor of the transcription of *E6* and *E7* oncogenes (Goodwin and DiMaio, 2000; zur Hausen, 2000). The best-known interactions of E6 and E7 are the interactions with p53 and Rb, respectively. These interactions are believed to deregulate cell-cycle control, the response to DNA damage and thus contribute to tumorigenesis. However, pleiotropic interactions of E6 and E7 have been identified and documented extensively recently (Jones and Wells, 2006). The detection of intracellular Notch in cervical cancers in the mid-90s was of interest beyond the context of neoplasias as such forms of Notch are extremely hard to detect. An intense debate at that point was built around the issue of whether Notch signaling was dependent on regulated cleavage of the receptor and localization of this fragment in the nucleus. (Figures 1–3).

The genomic fragment of murine int-3 (*Notch-4*) gene identified from the MMTV screen (see above) was transfected into a mouse mammary epithelial cell line and was shown to markedly enhance growth in soft agar colony formation assays. Bishop and co-workers then showed that truncated alleles of Notch1 and Notch2 complement the function of adenovirus E1A in similar assays (Capobianco *et al*., 1997). The papillomavirus *E6* and *E7* oncogenes from the high-risk viruses share many similarities with adenovirus-transforming genes and are essential for cervical carcinogenesis. Thus, cooperative transformation assays were also carried out to investigate a possible interaction between Notch and E6 and E7 (Rangarajan *et al*., 2001). Interestingly, neither

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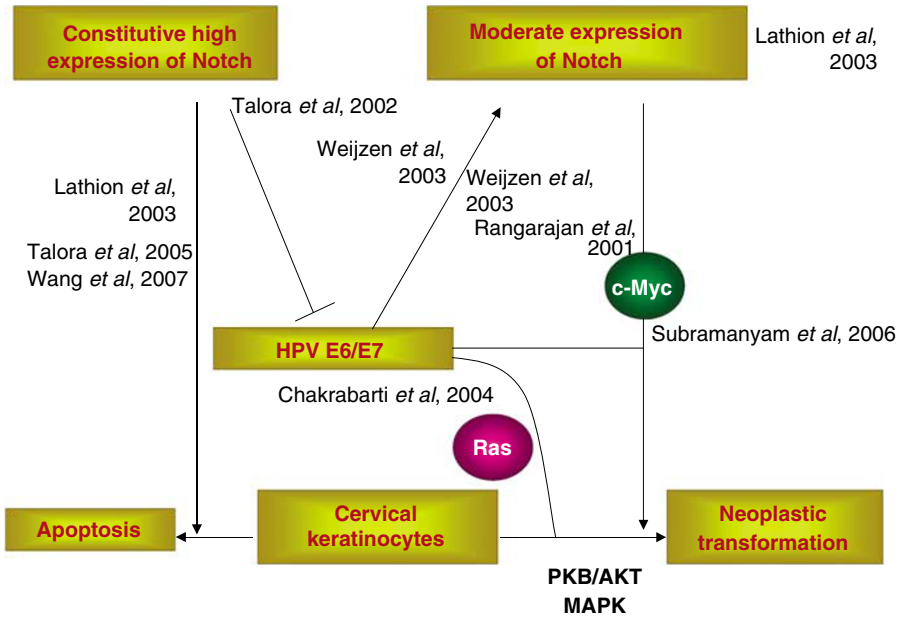

Figure 1 The role of Notch signaling in neoplastic transformation of cervical epithelium. Cervical keratinocytes undergo neoplastic transformation induced by human papillomavirus (*HPV*) oncogenes either by cooperating with Ras or Notch1. Moderate levels of Notch1 can upregulate c-Myc and activate PKB/Akt and induce transformation although exaggerated levels of activated Notch1 can induce apoptosis.

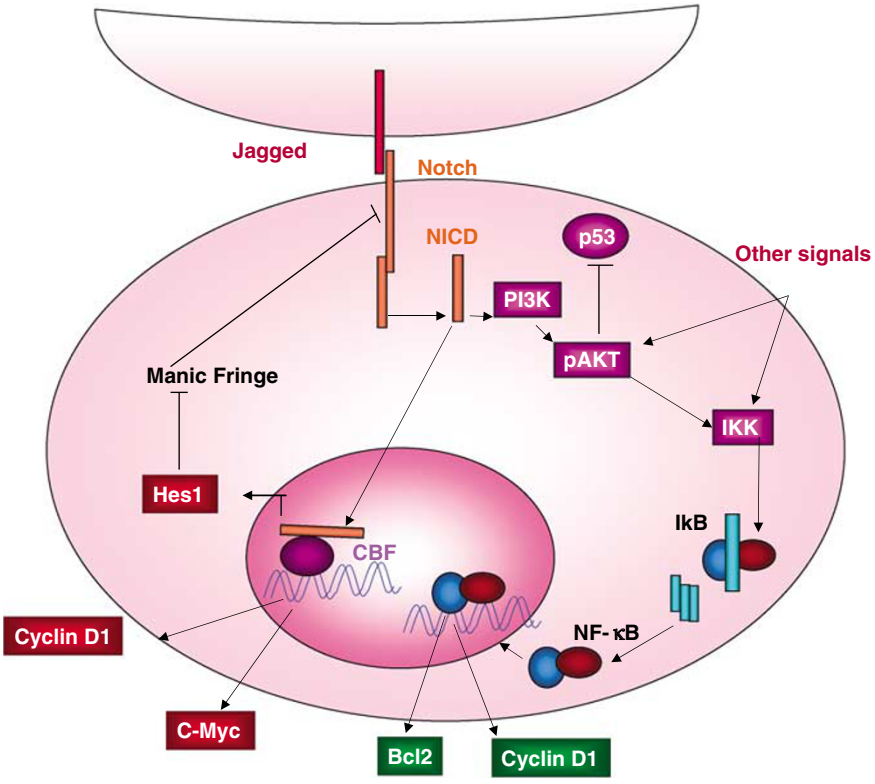

Figure 2 Jagged-mediated Notch signaling supports cervical carcinoma progression by C-protein binding factor (CBF)-dependent and -independent pathways. In the absence of detectable Notch1 mutations, cervical cancers are characterized by features of ligand-dependent activation of the pathway. There are pleiotropic effects attributed to activated PI3K/Akt that include inactivation of p53 and activation of nuclear factor (NF)-κB. The combined transcriptional activity of Notch1 and NF-κB may be convergent on the same target genes.

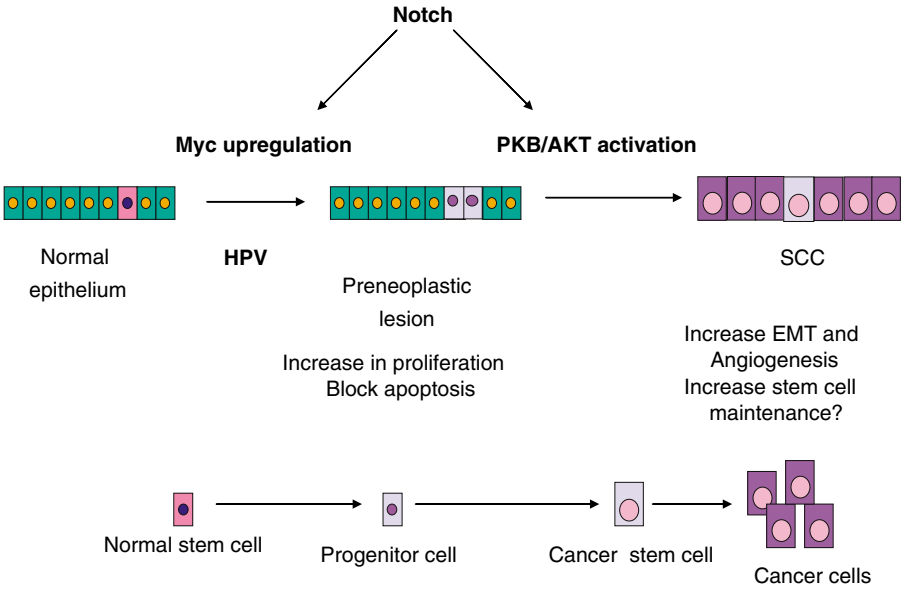

Figure 3 Notch supports malignant conversion in multiple ways. Notch by upregulating Myc or activating PKB/Akt pathway supports malignant conversion by increasing proliferation, blocking apoptosis, increasing epithelial–mesenchymal transition (EMT) and angiogenesis, and possibly by favoring self renewal of progenitor or the cancer stem cell pool.

E6 nor E7 were by themselves sufficient to generate cooperative transformation with truncated alleles of Notch1. However, in combination, the two oncogenes were able to cooperate with truncated alleles of Notch1 and give rise to soft agar colonies of HaCaT, an immortalized keratinocyte cell line. A subsequent report showed that these cells transfected with E6, E7 and Notch1 were able to generate xenograft tumors in nude mice (Chakrabarti *et al*., 2004). Peter Beard's laboratory extended these observations using primary human keratinocytes. They also reported a dose-dependent effect of Notch signaling and complex interactions with the regulation of HPV transcription (Lathion *et al*., 2003).

A variant of E6 (L83V), that has been shown to accumulate in the context of invasive cervical cancers in some studies, leads to a marked enhancement of both the *in vitro* soft agar and *in vivo* tumor forming capability of these cells. Interestingly, in parallel assays there was an inhibition of activated Ras-induced transformation by this E6 83aa variant (Chakrabarti *et al*., 2004). Collectively these two observations strengthen the notion that deregulated Notch signaling and not activated *Ras* alleles are associated with cervical cancer progression. These cancers are indeed marked by relatively low frequency of mutated *Ras* alleles.

The human *Notch1* gene was originally cloned from a translocation in human leukemia. More recently, 50% of T-cell acute lymphoblastic leukemias were found to harbor activating mutations in the Notch1 locus (Weng *et al*., 2004). A limited series of sequencing of *Notch1* alleles from human cervical cancer material focusing on the regions that have been implicated in the leukemias have not revealed any mutations (D Subramanyam and S Krishna, unpublished observations). In contrast, the expression patterns appear to favor a model that is dependent on ligand-dependent signaling. There is consistent expression of the Notch ligand Jagged1, coupled with diminished levels of Manic Fringe, a negative regulator of Jagged–Notch1 interactions (Veeraraghavalu *et al*., 2004). In addition, there are several observations that position a key role for this inverse expression of Jagged and Manic Fringe in the context of these cancers. An analysis of the W12 cell line derived from a patient harboring an HPV 16 infection revealed some striking insights into the patterns of Notch ligand and modifier expression. These cells upon *in vitro* passaging show a dramatic shift in the status of the papillomavirus genome status with an integration of the episomes (Pett *et al*., 2004). There is a consequent acquisition of features of more advanced neoplasias analogous to the progression of precursor lesions. On organotypic raft, the early passage cells exhibit high levels of Manic Fringe with low levels of Jagged1 (Veeraraghavalu *et al*., 2004). The reciprocal pattern is seen in the late passage cells consistent with switches in Notch activation states. This switch also correlates with the progression of neoplastic features as the early passage W12 cells are nontumorigenic whereas the later passage cells form xenografts in nude mice (Pett *et al*., 2004). Canonical Notch signaling involves an association with CSL family of transcription factors that can both repress and activate gene expression. Consistent with a negative feedback loop that regulates the expression of Manic Fringe, CSL-dependent reporters were upregulated in late passage W12 cells. Correspondingly, HES binding sites have also been identified in the upstream regulatory region of Manic Fringe (Veeraraghavalu *et al*., 2004). These observations were then

extended to an established human cervical tumor-derived cell line CaSki, where it was seen that an adenovirus expressing Manic Fringe was able to inhibit xenograft growth.

Anchorage-independent growth (anoikis) is one of the hallmarks of transformed cells. The functional contribution of activated Notch signaling in the context of anchorage-independent growth has been analysed both in the context of adenovirus E1A and HPV E6 and E7-induced transformation. Cyclin D upregulation was identified as an important target in adenovirus E1A-induced transformation (Ronchini and Capobianco, 2001). Activation of PI3K/Akt was reported to contribute to transformation by Notch1 in the context of papillomavirus-mediated transformation by generating a survival signal (Rangarajan *et al*., 2001). This survival phenotype seen during epithelial transformation was further analysed in the context of the apoptotic function of *p53* gene (Nair *et al*., 2003). The Notch-p53 interaction is clearly more complex than initially assumed given that p53 has been shown to regulate the expression of Notch1 in human keratinocytes (Yugawa *et al*., 2007). The Notch–PI3K–Akt link that was initially established in the context of anoikis resistance was then extended to the regulation of epithelial–mesenchymal transition (EMT; Veeraraghavalu *et al*., 2005). Interestingly, this interaction appears to mediate survival in diverse cells including T cells (Sade *et al*., 2004). More recently, cooperative transformation assays similar to those carried out with E6, E7 and Notch in HaCaT cells have identified c-Myc upregulation as an essential component of the cooperative transformation process. CaSki cells also show a similar pattern with a role for c-Myc (Subramanyam and Krishna, 2006). Collectively, these data have positioned a role for deregulated Notch signaling in driving epithelial transformation.

Other studies that have utilized techniques to block Notch RNA levels in nonepithelial and the CaSki cells have supported a role for Notch signaling in driving transformation in the background of *HPV* oncogenes (Lathion *et al*., 2003; Weijzen *et al*., 2003). The complexity of the role of Notch signaling in the context of human cervical cancers is highlighted by studies which have shown that high expression of Notch1 leads to growth arrest of cervical tumor-derived cells (Talora *et al*., 2005, 2002; Wang *et al*., 2007). This was interpreted as a tumor-suppressive role for Notch signaling in conjunction with the notion that Notch1 is actually downregulated during tumor progression. A later study on an extremely large series of clinical samples did not reveal any evidence of downregulation *per se* (Ramdass *et al*., 2007). In contrast the data broadly supported a persistent activation of the pathway in a ligand-dependent manner along with crosstalk with the nuclear factor (NF)-κB pathway.

A resolution of some of the apparently conflicting observations of Notch signaling in the context of human cervical cancers may emerge from an examination of the cancer stem cell paradigm in these tumors. An identification of stem cells and determining both their patterns of expression in terms of the Notch pathway and their susceptibility to modulation would help resolve some of these issues. Recent work in brain tumors has shown that the CD133+ cancer stem cells are modulated by Notch signaling (Fan *et al*., 2006). This would perhaps be a promising approach to evaluate in the context of cervical cancers. A second approach would be to undertake an analysis of transgenic mice that express papillomavirus oncogenes (Brake and Lambert, 2005) and examine the role of Notch signaling in cervical cancers *in situ*. Finally, given the role of Notch ligands in regulating peripheral T-cell responses, an integration of cancer development with the immune system would complete the picture in terms of all the interactions.

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References

Brake T, Lambert PF. (2005). Estrogen contributes to the onset, persistence, and malignant progression of cervical cancer in a human papillomavirus-transgenic mouse model. *Proc Natl Acad Sci USA* **102**: 2490–2495.

Capobianco AJ, Zagouras P, Blaumueller CM, Artavanis-Tsakonas S, Bishop JM. (1997). Neoplastic transformation by truncated alleles of human NOTCH1/TAN1 and NOTCH2. *Mol Cell Biol* **17**: 6265–6273.

Chakrabarti O, Veeraraghavalu K, Tergaonkar V, Liu Y, Androphy EJ, Stanley MA *et al*. (2004). Human papillomavirus type 16 E6 amino acid 83 variants enhance E6-mediated MAPK signaling and differentially regulate tumorigenesis by notch signaling and oncogenic Ras. *J Virol* **78**: 5934–5945.

Daniel B, Rangarajan A, Mukherjee G, Vallikad E, Krishna S. (1997). The link between integration and expression of human papillomavirus type 16 genomes and cellular changes in the evolution of cervical intraepithelial neoplastic lesions. *J Gen Virol* **78**(Part 5): 1095–1101.

Fan X, Matsui W, Khaki L, Stearns D, Chun J, Li YM *et al*. (2006). Notch pathway inhibition depletes stem-like cells and blocks engraftment in embryonal brain tumors. *Cancer Res* **66**: 7445–7452.

Goodwin EC, DiMaio D. (2000). Repression of human papillomavirus oncogenes in HeLa cervical carcinoma cells causes the orderly reactivation of dormant tumor suppressor pathways. *Proc Natl Acad Sci USA* **97**: 12513–12518.

Jones EE, Wells SI. (2006). Cervical cancer and human papillomaviruses: inactivation of retinoblastoma and other tumor suppressor pathways. *Curr Mol Med* **6**: 795–808.

Lathion S, Schaper J, Beard P, Raj K. (2003). Notch1 can contribute to viral-induced transformation of primary human keratinocytes. *Cancer Res* **63**: 8687–8694.

Nair P, Somasundaram K, Krishna S. (2003). Activated Notch1 inhibits p53-induced apoptosis and sustains transformation by human papillomavirus type 16 E6 and E7 oncogenes through a PI3K-PKB/Akt-dependent pathway. *J Virol* **77**: 7106–7112.

Pett MR, Alazawi WO, Roberts I, Dowen S, Smith DI, Stanley MA *et al*. (2004). Acquisition of high-level chromosomal instability is associated with integration of human papillomavirus type 16 in cervical keratinocytes. *Cancer Res* **64**: 1359–1368.

Ramdass B, Maliekal TT, Lakshmi S, Rehman M, Rema P, Nair P *et al*. (2007). Coexpression of Notch1 and NF-kappaB signaling pathway components in human cervical cancer progression. *Gynecol Oncol* **104**: 352–361.

Rangarajan A, Syal R, Selvarajah S, Chakrabarti O, Sarin A, Krishna S. (2001). Activated Notch1 signaling cooperates with papillomavirus oncogenes in transformation and generates resistance to apoptosis on matrix withdrawal through PKB/Akt. *Virology* **286**: 23–30.

Reid R. (1983). Genital warts and cervical cancer. II. Is human papillomavirus infection the trigger to cervical carcinogenesis? *Gynecol Oncol* **15**: 239–252.

Robbins J, Blondel BJ, Gallahan D, Callahan R. (1992). Mouse mammary tumor gene int-3: a member of the notch gene family transforms mammary epithelial cells. *J Virol* **66**: 2594–2599.

Ronchini C, Capobianco AJ. (2001). Induction of cyclin D1 transcription and CDK2 activity by Notch(ic): implication for cell cycle disruption in transformation by Notch(ic). *Mol Cell Biol* **21**: 5925–5934.

Sade H, Krishna S, Sarin A. (2004). The anti-apoptotic effect of Notch-1 requires p56lck-dependent, Akt/PKB-mediated signaling in T cells. *J Biol Chem* **279**: 2937–2944.

Subramanyam D, Krishna S. (2006). c-Myc substitutes for Notch1-CBF1 functions in cooperative transformation with papillomavirus oncogenes. *Virology* **347**: 191–198.

Talora C, Cialfi S, Segatto O, Morrone S, Kim Choi J, Frati L *et al*. (2005). Constitutively active Notch1 induces growth arrest of HPV-positive cervical cancer cells via separate signaling pathways. *Exp Cell Res* **305**: 343–354.

Talora C, Sgroi DC, Crum CP, Dotto GP. (2002). Specific down-modulation of Notch1 signaling in cervical cancer cells is required for sustained HPV-E6/E7 expression and late steps of malignant transformation. *Genes Dev* **16**: 2252–2263.

Veeraraghavalu K, Pett M, Kumar RV, Nair P, Rangarajan A, Stanley MA *et al*. (2004). Papillomavirus-mediated neoplastic progression is associated with reciprocal changes in JAGGED1 and manic fringe expression linked to notch activation. *J Virol* **78**: 8687–8700.

Veeraraghavalu K, Subbaiah VK, Srivastava S, Chakrabarti O, Syal R, Krishna S. (2005). Complementation of human papillomavirus type 16 E6 and E7 by Jagged1-specific Notch1-phosphatidylinositol 3-kinase signaling involves pleiotropic oncogenic functions independent of CBF1;Su(H);Lag-1 activation. *J Virol* **79**: 7889–7898.

Wang L, Qin H, Chen B, Xin X, Li J, Han H. (2007). Overexpressed active Notch1 induces cell growth arrest of HeLa cervical carcinoma cells. *Int J Gynecol Cancer* **17**: 1283–1292.

Weijzen S, Zlobin A, Braid M, Miele L, Kast WM. (2003). HPV16 E6 and E7 oncoproteins regulate Notch-1 expression and cooperate to induce transformation. *J Cell Physiol* **194**: 356–362.

Weng AP, Ferrando AA, Lee W, Morris JPt, Silverman LB, Sanchez-Irizarry C *et al*. (2004). Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* **306**: 269–271.

Yugawa T, Handa K, Narisawa-Saito M, Ohno S, Fujita M, Kiyono T. (2007). Regulation of Notch1 gene expression by p53 in epithelial cells. *Mol Cell Biol* **27**: 3732–3742.

Zagouras P, Stifani S, Blaumueller CM, Carcangiu ML, Artavanis-Tsakonas S. (1995). Alterations in Notch signaling in neoplastic lesions of the human cervix. *Proc Natl Acad Sci USA* **92**: 6414–6418.

zur Hausen H. (2000). Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. *J Natl Cancer Inst* **92**: 690–698.

zur Hausen H. (2002). Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* **2**: 342–350.