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Notch tumor suppressor function

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Abstract

Cancer development results from deregulated control of stem cell populations and alterations in their surrounding environment. Notch signaling is an important form of direct cell-cell communication involved in cell fate determination, stem cell potential and lineage commitment. The biological function of this pathway is critically context-dependent. Here we review the pro-differentiation role and tumor suppressing function of this pathway, as revealed by loss of function in keratinocytes and skin, downstream of p53 and in cross-connection with other determinants of stem cell potential and/or tumor formation, like p63 and Rho/CDC42 effectors. The possibility that Notch signaling elicits a duality of signals, involved in growth/differentiation control and cell survival will be discussed, in the context of novel approaches for cancer therapy.

Keywords

cancer stem cells; p53; p63; Rho signaling; epigenetics; keratinocytes; carcinogenesis; cancer therapy

Introduction

Cancer development is the combined result of deregulated stem cell populations and alterations in their surrounding environment. Cell and tissue homeostasis is achieved by a complex interplay of signaling pathways, coordinating behavior of a cell with its neighbors in a closely integrated fashion. Thus, the biological function of individual cell regulatory pathways can only be understood within the specific cells and tissues in which they operate. This is particularly relevant for Notch signaling, and its highly context-dependent function in different cell types and, within the same cell type, at different developmental stages and under normal versus pathological conditions. At the basis of the context-dependent function of Notch, three determinants of specificity can be envisaged: a) cross-talk of Notch signaling with cell-type specific regulatory molecules; b) cell-type specific cross-talk of Notch with other regulatory pathways, which are themselves not cell type specific; c) cell type- and tissue-specific global organization, to which Notch contributes and in the context of which it functions.

In mammalian systems, Notch activation is generally held to promote cancer development, while it can also play an opposite role. Biologically, this is not surprising, in view of the opposite role that Notch can play in enhancing stem cell potential and suppressing differentiation along certain lineages, while promoting commitment to others (Artavanis-Tsakonas *et al.*, 1999).

The focus of this review is on the function of Notch in the skin/keratinocyte system, where loss of function experiments have unequivocally demonstrated a tumor suppressing function

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of this pathway in both mice and humans (Nicolas *et al.*, 2003; Proweller *et al.*, 2006; Lefort *et al.*, 2007). Similar suppressing function is also possible for other tumor types, as suggested by the decreased expression and/or activity of endogenous Notch receptors in these tumors and the growth inhibitory effects exerted by increased Notch activity in the corresponding cells (Sriuranpong *et al.*, 2001; Sriuranpong *et al.*, 2002; Kunnimalaiyaan *et al.*, 2006; Chen *et al.*, 2007; Zheng *et al.*, 2007). Importantly, however, besides control of stem cell renewal and differentiation, Notch signaling plays a pivotal role in cell survival, in a manner that can deeply influence the final outcome in tumor development. As discussed at the end, this limits the dichotomy between tumor-promoting and suppressing function, and has important implications for Notch signaling as a multifaceted target for novel approaches of cancer therapy.

Notch as a negative regulator of keratinocyte stem cell potential and inducer of differentiation

Notch signaling involves a direct form of cell-cell communication, whereby non-diffusible ligands expressed at the surface of a cell engage and activate cognate receptors on its neighbor (Bray, 2006). The best characterized “canonical” pathway of Notch activation involves proteolytic cleavage and translocation of the cytoplasmic domain of the receptor to the nucleus, where it associates with the DNA binding protein CSL converting it from a repressor into an activator of transcription (Mumm and Kopan, 2000; Lai, 2002). However, direct binding of Notch to a second ancillary protein, Mastermind-like 1–3 (MamL 1–3), is required for elevated levels of CSL-dependent transcriptional activation through recruitment of further transcriptional co-activators such as p300 (Petcherski and Kimble, 2000; Wu *et al.*, 2000; Oswald *et al.*, 2001). The best characterized targets of Notch/CBF-1/MamL mediated activation are members of the HES and HERP families of bHLH transcriptional repressors (Iso *et al.*, 2003). However, a number of other direct targets of Notch/CBF-1 transcription have been identified, which can be induced by Notch activation in a cell type specific manner.

Direct cell-cell communication by Notch is employed for “lateral inhibition”, a process whereby negative cross-regulation between Notch and its ligand(s) in equipotent progenitors produces cells with divergent cell fates (Bray, 2006). Notch and its ligands can also be part of a positive feedback mechanism; here, Notch-dependent up-regulation of ligand expression and consequent reinforcement of Notch activation are involved in the coordination of differentiation along the same lineage. Both mechanisms are likely to operate in mammalian skin. In human epidermis, localized expression of the Notch-ligand Delta in putative “stem cells” has been proposed to induce commitment of neighboring Notch1-expressing cells to a “transit amplifying” phenotype, a scenario reminiscent of lateral inhibition (Lowell *et al.*, 2000). On the other hand, expression of the Jagged 1/2 ligands and Notch 1 and 2 receptors increases coincidentally in differentiating keratinocytes of the supra-basal layers of the epidermis, suggesting that they are part of a positive reinforcement mechanism required for synchronization of differentiation and epidermal border formation (Luo *et al.*, 1997; Rangarajan *et al.*, 2001). In fact, an essential role of Notch1 in this process was demonstrated by the fact that mice with a conditional keratinocyte-specific deletion of this gene show suprabasal expression of integrins that are usually confined to the basal layer of the epidermis, and exhibit overlapping basal and suprabasal marker expression (Rangarajan *et al.*, 2001). A more complex role of Notch signaling in the coordinate control of keratinocyte differentiation along the various hair follicle lineages versus the interfollicular cell fate has been unveiled by the concomitant deletion of the Notch1 and 2 and/or RBP-Jκ genes (Yamamoto *et al.*, 2003; Pan *et al.*, 2004; Blanpain *et al.*, 2006).

The biochemical mechanisms mediating the pro-differentiation function of Notch in keratinocytes have been studied intensively. The cyclin/CDK inhibitor $p21^{WAF1/Cip1}$ has

emerged as an important target under positive Notch1 control in mouse keratinocytes, both in culture and in the intact epidermis *in vivo* (Rangarajan *et al.*, 2001; Nicolas *et al.*, 2003; Lee *et al.*, 2007), which mediates the growth inhibitory effects of Notch1 activation (Rangarajan *et al.*, 2001). Increased Notch1 activity leads to increased p21 expression through two convergent mechanisms that involve direct binding of the RBP-Jκ protein to the p21 protein (Rangarajan *et al.*, 2001) and RBP-Jκ-dependent activation of the calcineurin/NFAT pathway (Mammucari *et al.*, 2005). Importantly, in keratinocytes of hair follicles, p21 expression appears to be under opposite Notch1 control than in the interfollicular epidermis (Lee *et al.*, 2007), consistent with the more complex function of this pathway in hair follicles, which can be attributed, at least in part, to distant growth-factor mediated interactions (Lin *et al.*, 2000).

Besides cell cycle withdrawal, two other essential aspects of keratinocyte differentiation elicited by Notch activation are down-regulation of integrins of the basal layer and induction of differentiation markers of the supra-basal layers (keratin 1/10, involucrin) (Rangarajan *et al.*, 2001; Nickoloff *et al.*, 2002b). Down-modulation of integrins by Notch activation is not due to a direct RBP-Jκ dependent mechanism (Rangarajan *et al.*, 2001). As for the induction of supra-basal differentiation markers, a recent report that they are under positive RBP-Jκ/HES1 control (Blanpain *et al.*, 2006) is not consistent with other publications showing an inhibitory rather than inducing function of HES1 on these markers, both in cultured keratinocytes and in the intact skin (Nguyen *et al.*, 2006; Moriyama *et al.*, 2008).

Besides intracellular regulatory mechanisms, Notch signaling is likely to impinge on keratinocyte growth/differentiation control and skin homeostasis more indirectly, via modulation of growth factor and cytokine production. In fact, another important consequence of Notch activation in keratinocytes is induction of the canonical as well as non-canonical NF-κB pathway (Nickoloff *et al.*, 2002a; Lefort *et al.*, 2007; Osipo *et al.*, 2008). The role of NF-κB dependent modulation of cytokine production by Notch signaling has not been explored in great detail but it is an area of likely importance. Another major class of genes related to cytokine production that are modulated by Notch in keratinocytes are genes involved in the interferon response (Nguyen *et al.*, 2006; Perera *et al.*, 2006). Importantly, Notch activation in keratinocytes causes selective suppression of some interferon responsive genes, while inducing others, pointing to the existence of a novel mechanism for the fine tuning of these genes, which may be of particular significance for modulation of growth-differentiation control. In fact, several of the interferon responsive genes under Notch control in keratinocytes (Nguyen *et al.*, 2006), including some diffusible cytokines, have been previously implicated in growth control, apoptosis and/or tumorigenesis (Ghosh *et al.*, 2001; Carpten *et al.*, 2002; Wasylyk *et al.*, 2002; Zhang and Pagano, 2002).

Significant differences are known to exist between growth control of cells of mouse versus human origin (Rangarajan *et al.*, 2004). These extend to keratinocytes and their different response to Notch activation (Nguyen *et al.*, 2006). Notch1 activation triggers direct cell cycle withdrawal of mouse primary keratinocytes (Rangarajan *et al.*, 2001); in keratinocytes of human origin it has less immediate effects, causing these cells to replicate for a limited number of times with a subsequent loss of clonogenic potential (Lowell *et al.*, 2000; Nguyen *et al.*, 2006; Lefort *et al.*, 2007). At the transcriptional level, only a restricted subset of genes is similarly controlled by Notch1 activation in the two types of cells (Nguyen *et al.*, 2006). Among the commonly regulated genes is p63 (Nguyen *et al.*, 2006), a p53 family member with a selective essential function in keratinocyte cell fate determination and/or stem cell maintenance (Mills *et al.*, 1999; Yang *et al.*, 1999). Besides its role in skin development, p63 is likely to play a significant function after birth, preventing differentiation (King *et al.*, 2003; Koster and Roop, 2004) and premature senescence (Keyes *et al.*, 2005). Importantly, elevated expression of this gene has also been associated with a variety of epithelial tumors, including squamous cell carcinomas (Parsa *et al.*, 1999; Pellegrini *et al.*, 2001; Westfall and Pietenpol, 2004), in

which Notch1 expression and activity are downmodulated (Lefort *et al.*, 2007), suggesting that the two are linked. In fact, in keratinocytes of both murine and human origin, there is a reciprocal negative feedback loop between Notch and p63. On one hand, p63 expression is suppressed by Notch activation through a novel mechanism involving the selective modulation of interferon responsive genes mentioned above (Nguyen *et al.*, 2006). On the other hand, persistently elevated p63 expression counteracts the growth inhibitory effects of Notch activation, while synergizing with other aspects of Notch function involved in the early stages of differentiation (Nguyen *et al.*, 2006; Truong *et al.*, 2006; Okuyama *et al.*, 2007).

Besides p63, Smad proteins have also been shown to functionally and biochemically interact with activated Notch1 (Blokzijl *et al.*, 2003; Itoh *et al.*, 2004; Zavadil *et al.*, 2004). This can be relevant for the tumor suppressing function discussed below, as recent results point to the synergism of Notch and TGF-β signaling in keratinocyte growth inhibition, with $p21^{WAF1/Cip1}$ as an important downstream target (Niimi *et al.*, 2007).

Tumor suppressor function of Notch in mouse keratinocyte tumor development

Mouse skin is a classical experimental system for epithelial tumor development, where the notion of multistep chemical carcinogenesis has been originally defined (Yuspa, 1991). Notch signaling plays an important tumor suppressing function in this context. In fact, keratinocyte-specific deletion of the Notch1 gene results in a substantially increased susceptibility to chemical or *ras*-induced skin carcinogenesis (Nicolas *et al.*, 2003), with similar effects being observed after deletion of the Delta like 1 (Estrach *et al.*, 2008) or γ-secretase (Xia *et al.*, 2001; Li *et al.*, 2007) genes, or expression of a dominant negative peptide suppressing Notch/CSL dependent transcription (Proweller *et al.*, 2006) (MAM51, as also discussed further below).

Importantly, loss of the Notch1 gene mirrors the effects of loss of $p21^{WAF1/Cip1}$ on both keratinocyte stem cell populations (Topley *et al.*, 1999) as well as *ras*- (Missero *et al.*, 1996) and chemically induced skin carcinogenesis (Philipp *et al.*, 1999; Topley *et al.*, 1999; Weinberg *et al.*, 1999). The common biological function of Notch1 and p21 as negative regulators of keratinocyte self renewal and tumorigenesis can be explained, at least in part, by suppression of *Wnt* signaling (Nicolas *et al.*, 2003; Devgan *et al.*, 2005). Activation of the *Wnt*/β-catenin pathway has been associated with keratinocyte stem cell populations (Zhu and Watt, 1999) and keratinocyte-derived tumors (Gat *et al.*, 1998), while, conversely, β-catenin gene deletion results in decreased skin cancer stem cell populations (Malanchi *et al.*, 2008). Genetic analysis in developmental model systems has pointed to a complex cross-talk between the Notch and Wnt signaling pathways, which can occur at multiple levels (Couso and Martinez Arias, 1994; Axelrod *et al.*, 1996; Galceran *et al.*, 2004; Hofmann *et al.*, 2004). In mouse keratinocytes and skin, Notch was found to negatively regulate *Wnt* signaling through a novel mechanism involving down-modulation of *Wnt* ligand gene expression, specifically and *Wnt4*. Importantly, p21 is a key mediator of the negative regulation of *Wnt4* expression, binding to the promoter region of this gene and functioning downstream of Notch, at the transcription-chromatin level and separately from effects on the cell cycle (Devgan *et al.*, 2005).

Besides $p21^{WAF1/Cip1}$ and Wnts, Notch signaling has been shown to affect other molecules and pathways with a key regulatory function in keratinocyte tumor development. Unlike p21 −/− mice, mice with the Notch1 deletion develop also spontaneous skin tumors in various parts of the body, with histological features similar to those of basal cell carcinomas (BCCs) (Nicolas *et al.*, 2003). Consistent with this phenotype, Notch1 deficiency is associated with increased and sustained expression of Gli2, a downstream target of the sonic hedgehog (SHH) signaling pathway that has been causally linked to BCC tumor formation (Nicolas *et al.*, 2003). Another

distinguishing characteristic of mice with a keratinocyte-specific deletion of the Notch1 gene is the development of extensive hyperplasia and keratinization of the corneal epithelium that results in opaque plaque formation and blindness (Nicolas *et al.*, 2003). The underlying mechanism has been linked to defective vitamin A metabolism (Vauclair *et al.*, 2007), whose role in keratinocyte tumor formation has been previously intensely investigated (Altucci and Gronemeyer, 2001).

Role of Notch1 in human keratinocyte tumor suppression

The genetic studies discussed above demonstrate that Notch signaling, and more specifically Notch1, acts as a tumor suppressor in mouse skin. A key question is whether this tumor suppressing function is a peculiarity of the mouse skin system or applies also to the human situation. This is an important issue, given the tumor promoting function commonly attributed to Notch signaling and its clinical implications (Miele *et al.*, 2006). Even for human keratinocytes, as discussed in an accompanying review (Krishna), it was originally proposed that Notch1 activation cooperates with HPV-induced cervical carcinogenesis, through a mechanism involving an increase in cell survival (Rangarajan *et al.*, 2001; Nair *et al.*, 2003). This is consistent with the original finding (Zagouras *et al.*, 1995), confirmed by later reports ((Ramdass *et al.*, 2007) and refs. therein), that Notch1 receptor expression is elevated in cervical carcinomas. However, in light of the normal pro-differentiation function of Notch activation in normal keratinocytes (Lowell *et al.*, 2000; Rangarajan *et al.*, 2001; Nickoloff *et al.*, 2002a; Nguyen *et al.*, 2006; Lefort *et al.*, 2007), elevated expression of the Notch1 receptor in cervical carcinomas could also be interpreted as a marker of differentiation, expressed in the more differentiated parts of these tumors. Consistent with this interpretation is the heterogeneous pattern of Notch1 expression, decreased rather than increased in the less differentiated areas of aggressive cervical cancer (Talora *et al.*, 2002), and the fact that HPV-positive cervical carcinoma cell lines express significantly lower levels of the Notch1 protein than corresponding normal primary keratinocytes (Talora *et al.*, 2002; Yugawa *et al.*, 2007). Such down-modulation of Notch1 expression and activity may be required for tumor development, as expression of activated Notch1 in cervical cancer cells results in drastic growth suppression (Talora *et al.*, 2002; Wang *et al.*, 2007; Yao *et al.*, 2007). This is not due to a-specific toxic effects, but can be explained by suppression of HPV oncoprotein expression, through inhibition of endogeous AP-1 activity (Talora *et al.*, 2002), and/or additional more indirect mechanisms (Talora *et al.*, 2005; Yao *et al.*, 2007).

As in cervical carcinoma cells, the proliferative potential of primary human keratinocytes is suppressed by increased Notch activity (Lowell *et al.*, 2000; Nguyen *et al.*, 2006; Lefort *et al.*, 2007). To assess whether suppression of endogenous Notch signaling has converse tumor-promoting effects, two complementary approaches were undertaken. Expression of a 51 amino acid peptide corresponding to the N-terminus of the Mastermind-like 1 protein (MAM51) provides an effective way to suppress Notch/CBF-1-dependent transcription (Weng *et al.*, 2003). This method was previously used to suppress Notch signaling and inhibit tumorigenic behavior of T cell lymphomas as well as melanomas (Weng *et al.*, 2003; Maillard *et al.*, 2004; Balint *et al.*, 2005). In sharp contrast, this same approach increased dramatically the susceptibility of primary human keratinocytes to transformation by a *ras* oncogene, with formation of tumors closely resembling clinically aggressive SCCs (Lefort *et al.*, 2007). As a second approach, *ras*-expressing human keratinocytes were tested by skin-reconstitution assays onto mice, under conditions that result, under normal conditions, in well stratified epidermis (Dotto *et al.*, 1989). Under control conditions, the *ras* expressing keratinocytes produced a hyperplastic epithelium with only limited sites of invasion. By contrast, treatment with a γ-secretase inhibitor, to suppress endogenous Notch activation, resulted in aggressive tumor formation (Lefort *et al.*, 2007).

Previous studies have reported oncogenic conversion of primary human keratinocytes by concomitant expression of oncogenic *ras* and suppression of NF-κB, through a mechanism that promotes growth through TNF/JNK activity (Dajee *et al.*, 2003; Zhang *et al.*, 2004). Since Notch activation leads to induction of NF-κB (Nickoloff *et al.*, 2002a; Nguyen *et al.*, 2006; Shin *et al.*, 2006), an attractive possibility was that the tumor suppressing function of Notch in keratinocytes is mediated by NF-κB. However, while suppressing "canonical" CBF-1 dependent transcription, expression of the MAM51 peptide did not affect NF-κB activity and had no direct growth promoting effects on the total keratinocyte cell population (Lefort *et al.*, 2007).

As pointed out in a recent review (Merlo *et al.*, 2006), increased growth potential and/or "immortalization" are not necessary characteristics of the majority of cells in primary tumors. What may be more relevant instead are "cancer stem cell populations", representing a minor, possibly slow cycling fraction of cells that give rise to all other heterogeneous tumor cell populations (Merlo *et al.*, 2006). Consistent with this possibility, Notch suppression causes the expansion, over time, of a minor subpopulation of keratinocytes with high self-renewing potential (Lefort *et al.*, 2007). In this context, the negative cross-talk between Notch1 and p63 discussed above can play an important role (Nguyen *et al.*, 2006). However, p63 expression is down-regulated by Notch activation through a CBF-1 and Hes-independent mechanism not affected by MAM51 expression (Nguyen *et al.*, 2006; Lefort *et al.*, 2007).

Like p63, small GTPases of the Rho, Rac and CD42 families have been recently implicated in keratinocyte cell fate determination and/or stem cell potential (Xu *et al.*, 2003; Benitah *et al.*, 2005; Dotto and Cotsarelis, 2005; Wu *et al.*, 2006), and increased Rho signaling has been connected with tumor progression (Sahai and Marshall, 2002). Importantly, no genetic mutations have been uncovered which are responsible for activation of this pathway in tumors. Searching for "canonical" CBF-1 dependent mechanisms involved in keratinocyte tumor suppression, the ROCK1/2 and MRCKα kinases, two key effectors of Rho GTPases over-expressed in tumors, were found to be under negative Notch/HES control (Lefort *et al.*, 2007). Importantly, the combined knockdown of these kinases could counteract the effects of Notch suppression in keratinocytes both *in vitro* and *in vivo*, pointing to an inverse relationship between Notch and Rho signaling in control of stem cell potential and tumorigenesis (Lefort *et al.*, 2007).

Notch 1 is a tumor suppressor gene under direct p53 control

Tumor suppressor genes are classically defined as genes whose mutation or loss are required for tumor development (Hahn and Weinberg, 2002). However, the importance of epigenetic mechanisms for down-modulation of negative growth regulatory genes and tumorigenesis is increasingly recognized (Gius *et al.*, 2004). Consistent with the tumor suppressing function of Notch signaling in keratinocytes, Notch activity is significantly reduced in the two major types of non-melanoma skin cancer, squamous cell carcinomas (SCCs) and basal cell carcinomas (BCCs) (Thelu *et al.*, 2002; Lefort *et al.*, 2007). Expression of the Notch1 gene itself is down-regulated, at the transcriptional level, with less pronounced effects for Notch2.

Surprisingly little is known of transcriptional control mechanisms of Notch receptor genes, although this can be an important mode of regulation of Notch activity. A recent global survey of the human genome pointed to the Notch1 promoter as a possible p53 binding target (Wei *et al.*, 2006). Given the high frequency of p53 mutations in skin SCCs (http://p53.free.fr), an attractive possibility was that down-modulation of Notch1 expression occurring in these tumors is due, at least in part, to compromised p53 function. In fact, in primary human keratinocytes, endogenous p53 binds to the Notch1 promoter and p53 knock-down in these cells results in down-modulation of Notch1 expression (Lefort *et al.*, 2007; Mandinova *et al.*, 2008).

Conversely, increased p53 levels, by either exogenous expression or stabilization of the endogenous protein (via MDM2 inhibition), leads to Notch1 up-regulation in normal keratinocytes and, to a substantial greater extent, in SCC cells (Lefort *et al.*, 2007).

In cervical carcinogenesis, the HPV E6/E7 oncogenes are expressed at low levels in low-grade intraepithelial neoplasias, while they are abundantly expressed in high-grade malignant lesions, with deregulated expression of these viral oncogenes being a key element for neoplastic progression (zur Hausen, 2000). The major target of the viral E6 protein is p53, which is targeted by E6 for degradation. Elegant genetic and biochemical studies showed that E6-dependent degradation of p53 is responsible for the strongly reduced levels of Notch1 expression in HPV-positive cervical carcinoma cells (Yugawa *et al.*, 2007). Even in this case p53 was found to regulate specifically Notch1 expression, with little or no effect on Notch2 (Yugawa *et al.*, 2007).

As p53 and p63 share a common DNA recognition sequence (Yang *et al.*, 1998), an attractive possibility is that p63 can also participate in control of Notch1 gene transcription. Consistent with this possibility is the lack of Notch1 expression in the epidermis of mice with p63 deletion during development (Laurikkala *et al.*, 2006). However, in mature keratinocytes, p63 counteracts downstream Notch signaling (Nguyen *et al.*, 2006) with little or no effect on Notch1 gene expression ((Yugawa *et al.*, 2007); Kolev et al., submitted).

Besides keratinocytes, similar regulation of Notch1 expression by p53 occurs in lung and prostate cancer cells, other cell types in which increased Notch signaling causes growth inhibition with possible tumor suppressing effects ((Alimirah *et al.*, 2007; Yugawa *et al.*, 2007); Lefort et al, submitted). In contrast, no such regulation was observed in colon carcinoma cells or fibroblast-derived cell lines, pointing to a likely interplay between p53 and other cell type-specific regulators in control of Notch1 gene transcription (Lefort *et al.*, 2007; Yugawa *et al.*, 2007).

Notch activity can also be controlled by p53 at the post-transcriptional level, as suggested by the finding that in T cells p53 can down-modulate Notch signaling through negative regulation of presenilin expression (Laws and Osborne, 2004). Conversely, Notch activity can mediate and/or modulate p53 function. In human cell types where it promotes tumor formation, Notch signaling was reported to act upstream of p53, suppressing its function (Beverly *et al.*, 2005; Mungamuri *et al.*, 2006). In keratinocytes, the finding that p53 is an upstream positive regulator of Notch1 raises the interesting possibility that p53 and Notch1 share common transcriptional targets, and the transcriptional response of one may depend, in part, on the other. This was shown to be indeed the case for at least one significant class of genes under Notch1 control, the ROCK1/2 and MRCKα kinases which are similarly regulated by increased p53 activity, in a manner which is, in the case of MRCKα, partially dependent on the Notch pathway (Lefort *et al.*, 2007).

Protective anti-apoptotic function of Notch signaling: at the cross-road between tumor promotion and suppression

UV light is a major etiological agent of skin aging and cancer (de Gruijl, 1999; Armstrong and Kricker, 2001). p53 plays a key role in the UV/DNA damage response of cells, controlling the decision between growth arrest and apoptosis (Latonen and Laiho, 2005; D'Errico *et al.*, 2007). The recent finding that Notch1 is a p53 target gene in keratinocytes pointed to the possibility that this gene is also implicated in the UVB/DNA damage response of these cells. In fact, UVB exposures of keratinocytes, in culture as well as in the skin, induces Notch1 gene expression in a p53-dependent manner, with similar induction being caused by genotoxic DNA-damaging agents (Yugawa *et al.*, 2007; Mandinova *et al.*, 2008). Such regulation is

physiologically relevant, as analysis of mice with a keratinocyte-specific deletion of the Notch1 gene as well as keratinocytes with Notch suppression showed that this gene plays a significant protective function against UVB-induced apoptosis (Mandinova *et al.*, 2008).

As for its role in growth/differentiation control, Notch signaling can exert either a pro- or anti-apoptotic function through multiple mechanisms that are highly cell- and context-dependent (Oswald *et al.*, 1998; Jehn *et al.*, 1999; Nair *et al.*, 2003; MacKenzie *et al.*, 2004; Oishi *et al.*, 2004; Sade *et al.*, 2004; Yang *et al.*, 2004; Kim *et al.*, 2005; Zweidler-McKay *et al.*, 2005). In the keratinocyte UVB response, the pro-survival function of Notch is linked to transcriptional down-modulation of FoxO3a, a key pro-apoptotic gene (Mandinova *et al.*, 2008). This occurs without detectable changes in PI3K/Akt-dependent phosphorylation that is a major form of FoxO3a regulation (Brunet *et al.*, 1999). Rather, the “canonical” Notch/HERP pathway is an important negative regulator of FoxO3a expression, through a mechanism involving binding of the HES/HERP/Tle transcription repressor complex to the FoxO3a promoter (Mandinova *et al.*, 2008).

FoxO3a is a transcription factor with multiple cellular functions, ranging, like Notch, from cell cycle control and differentiation to apoptosis (Burgering and Kops, 2002). In human keratinocytes, FoxO3a was recently found to control a subset of genes with cell cycle regulatory function ($p21^{WAF1/Cip1}$ and $p15^{INK4b}$), mediators of stress responses (like Gadd45α and β) and adaptive cell signaling (like Jagged1, CDC42EP3, OVOL1) (Gomis *et al.*, 2006), some of which are also under Notch control in these cells ((Nguyen *et al.*, 2006); our unpublished observations). Thus, an intriguing possibility is that FoxO3a participates in the already established cross-talk between Notch and other signaling pathways discussed in this review.

Irrespective of the detailed mechanism, the dual role of Notch in promoting commitment of keratinocytes to differentiation and, concomitantly, increasing their cell survival has important implications for possible therapeutic approaches based on targeting of this pathway. Based on our experimental evidence, we suggest that treatment with single general inhibitors of Notch activity (like γ-secretase inhibitors) can have detrimental tumor-promoting effects in the skin and possibly other organs where this pathway exerts a similar function. More promising approaches may be provided instead by selective inhibitors that can suppress the Notch pro-survival function while leaving intact or enhancing its ability to induce differentiation, or by a combination therapy based on Notch inhibitors in conjunction with other compounds with growth inhibitory and/or pro-differentiation activity.

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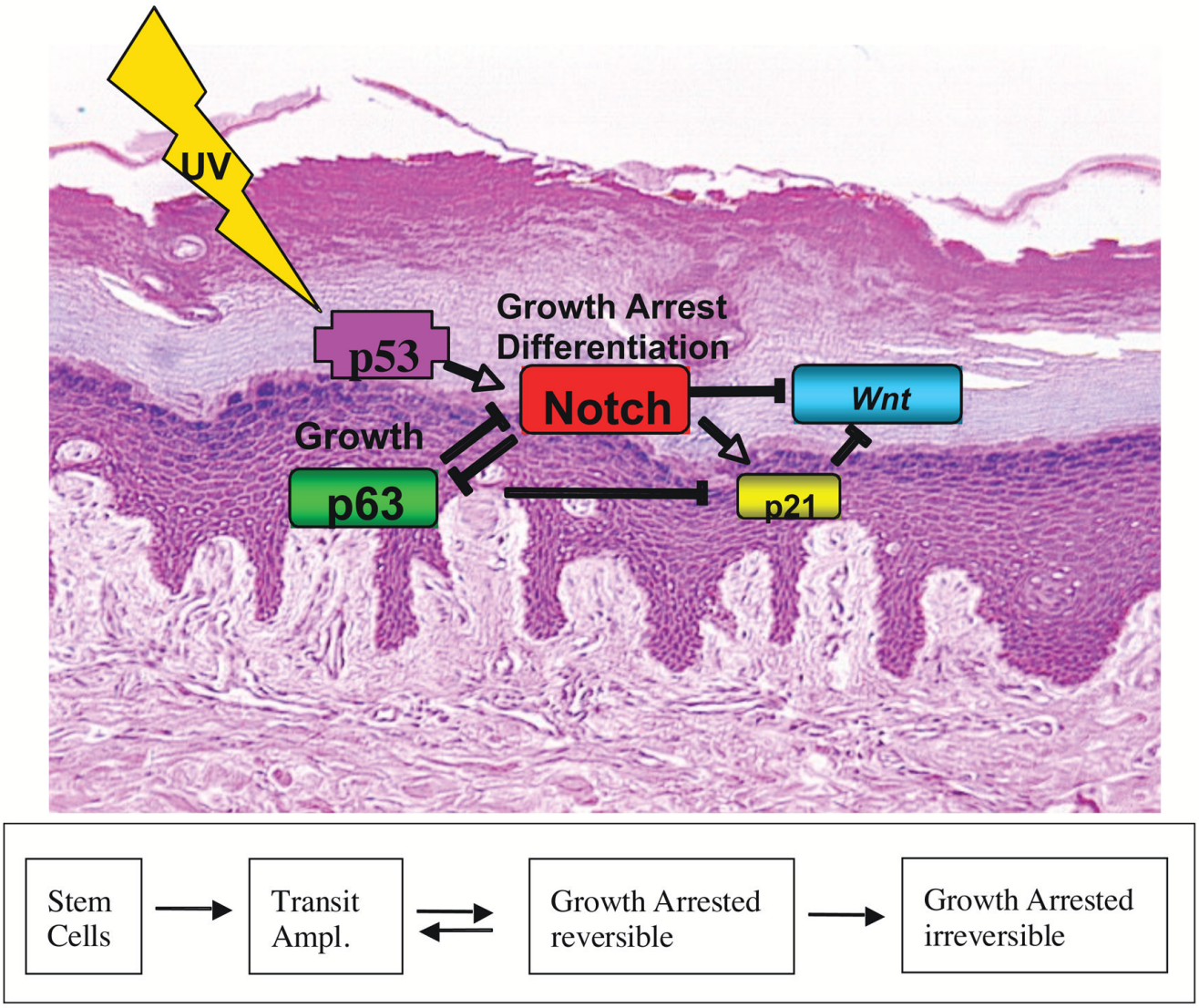

Figure 1. Cross-regulation of Notch with other key signaling pathways in keratinocyte stem self renewal, differentiation and/or tumorigenesis

A dynamic equilibrium exists in the skin among keratinocyte stem cells, transit amplifying populations, and cells that have exited the cell cycle and are committed reversibly versus irreversibly to differentiation. As discussed in the text, such equilibrium is controlled by an inverse gradient and reciprocal negative regulation of p63 expression and Notch activity in the lower versus upper epidermal layers. p63 suppresses Notch signaling in epidermal cells with high self-renewal potential, while synergizing with other aspects of Notch function in early stages of differentiation. Notch1 and $p21^{WAF1/Cip1}$, a "canonical" Notch target in keratinocytes, suppress *Wnt* ligand expression and signaling, and function as negative regulator of stem cell potential and tumorigenesis. In fact, UV light exposure is a major etiological agent of human skin cancer, and the Notch1 gene is a p53 target with a key role in human keratinocyte tumor suppression.

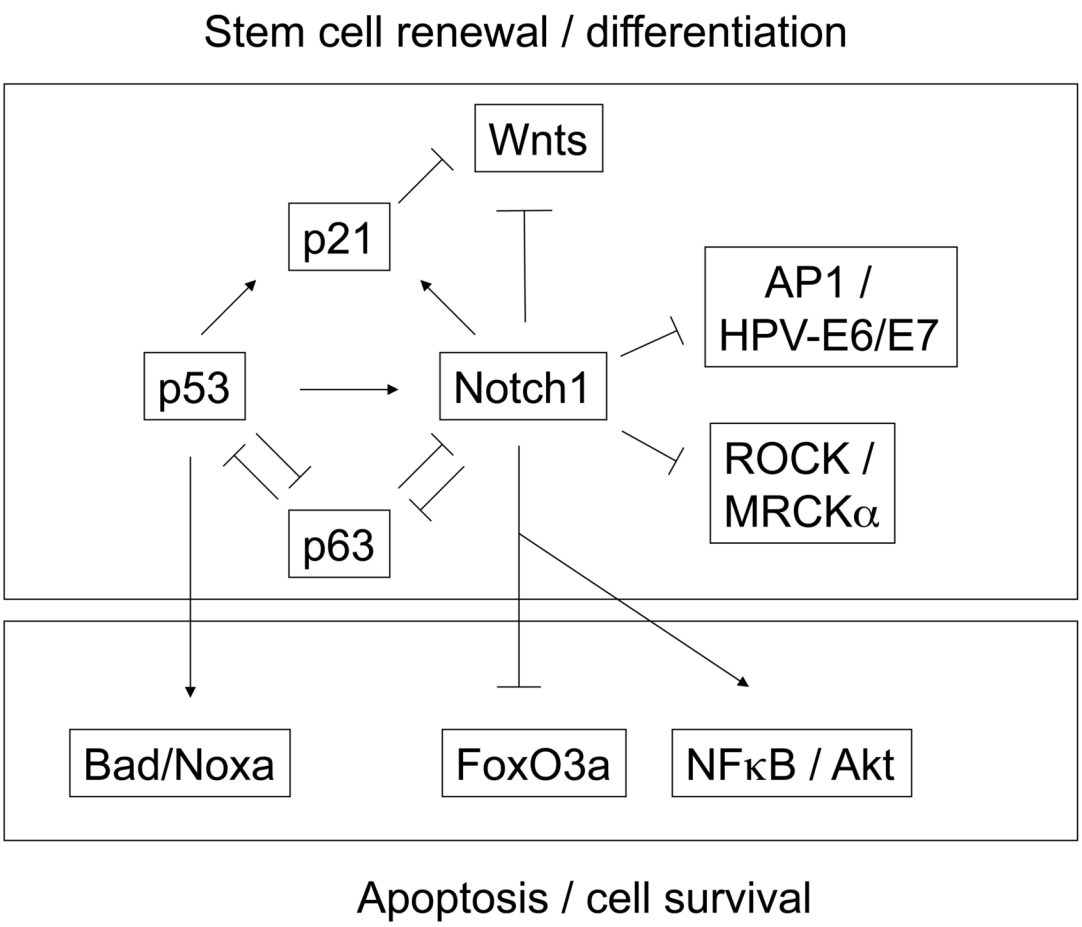

Figure 2. Duality of Notch functions in keratinocyte commitment to differentiation and cell survival

Notch signaling impacts on, and is regulated by a complex signaling network with an important role in skin homeostasis and tumor development. Like Notch, many other components of this network exert a duality of functions, impinging on keratinocyte growth/differentiation control as well as cell survival. This has potentially important implications for cancer therapy. An attractive approach could be the use of selective inhibitors, which can suppress the Notch pro-survival function while leaving intact or enhancing its ability to induce differentiation. Alternatively, Notch inhibitors, or inducers, could be used as part of a combination therapy based on in conjunction with other compounds with growth inhibitory and/or pro-apoptotic functions.