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Crosstalk of Notch with p53 and p63 in cancer growth control

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Abstract

Understanding the complexity of cancer depends on an elucidation of the underlying regulatory networks, at the cellular and intercellular levels and in their temporal dimension. This Opinion article focuses on the multilevel crosstalk between the Notch pathway and the p53 and p63 pathways. These two coordinated signalling modules are at the interface of external damaging signals and control of stem cell potential and differentiation. Positive or negative reciprocal regulation of the two pathways can vary with cell type and cancer stage. Therefore, selective or combined targeting of the two pathways could improve the efficacy and reduce the toxicity of cancer therapies.

Complex biological systems can be thought of as scale-free networks, with an uneven distribution of connections to key signalling nodes. Another central characteristic of these systems is that they are intrinsically robust, being capable of repair and recovery on perturbation¹. This robustness is the combined result of multiple factors. These include the redundancy of molecules with overlapping functions, feedback and feedforward loops, the modular organization of functionally coherent sets of molecules, such as those of individual signalling pathways, and more global integration at both intracellular and intercellular levels. Two major forms of network organization have been proposed: stress signalling networks, which are designed for transient responses to altered cell and tissue homeostasis, and developmental networks, which lead to more permanent cell fate decisions². Stress signalling networks are based on direct regulatory loops as well as transcription and signalling cascades for rapid on–off transitions. Developmental networks share the same basic motifs as stress signalling networks, but incorporate an additional level of complexity, with intersecting regulatory loops and transcription and signalling cascades that can lock on–off transitions in a more stable cell memory state. The focus of this Opinion article is the crosstalk between the Notch pathway and the p53 and p63 pathways, as a paradigm of

DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

CDKN2A|CCND1|EGFR|EWSR1|FLI1|FOXO3|IRF3|IRF7|MYC|Trp53

UniProtKB: <http://www.uniprot.org>

FBXW7|HES1|HEY1|JAG1|MAML1|MDM2|NOTCH1|p53|p63|p73|PTEN

FURTHER INFORMATION

G. Paolo Dotto's homepages: <http://www.unil.ch/ib/page9489.html> | <http://www.massgeneral.org/research/researchlab.aspx?id=1069>

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coordinated signalling modules in cancer development, at the interface between stress-generated signals and the control of stem cell potential and differentiation.

Crosstalk between the Notch pathway and the p53 and p63 pathways can occur at multiple levels, in a manner that is dependent on the overall network organization of the individual cell types or tissues in which they operate. I briefly overview the properties of the Notch, p53 and p63 signalling modules (FIGS 1,2), and refer the reader to recent excellent reviews for more detailed information on each of these pathways individually³⁻⁸. This is followed by an in-depth discussion of the cross-regulation between the two pathways at the level of Notch control of p53 expression and activity, reciprocal control of Notch by p53, cross-regulation between Notch and p63 (a p53 family member with a more selective role in development), and indirect cross-modulation between Notch and p53 and p63 by common downstream mediators and by interacting pathways. I conclude with a discussion of the effect that this integrated knowledge could have on new approaches to cancer therapy.

The Notch pathway

Developmental signalling pathways have a key role in the control of cancer development, as they impinge on target stem cell populations and their environment, organ morphogenesis and homeostasis. A restricted number of developmental signalling pathways is repeatedly used in various combinations and in a synergistic or opposite manner, giving rise to an extraordinary array of biological outcomes⁹. Activation of these pathways usually relies on the production of diffusible ligands — or morphogens — that function at a distance on their cognate receptors, which are expressed by other cells of the same or a different type. Notably different is the Notch signalling pathway, which relies instead on non-diffusible ligands, integral membrane proteins that engage and activate surface receptors of immediately adjacent cells³ (FIG. 1).

Notch signalling functions in lateral inhibition, a process whereby negative cross-regulation between Notch receptors and the Delta family of ligands in initially equipotent progenitors eventually produces an asymmetric distribution of cells with divergent cell fates³. Notch and the Jagged family of ligands can also be part of a positive feedback mechanism, involved in the coordinated control of differentiation along individual cell lineages, resulting in lateral induction and boundary formation³.

The biological outcome of Notch activation is highly context dependent. This also applies to the control of cancer development, which Notch activation is usually thought to promote^{10,11} but can, in at least some situations, abrogate¹². Biologically, this is not surprising, as Notch can either suppress or promote differentiation along certain cell lineages, and cancer is a probable result of deranged stem cell control or dedifferentiation of committed cell populations.

The p53 and p63 pathways

Although Notch activation can exert entirely opposite effects on basic cell fate decisions, such as proliferation, survival and differentiation decisions, p53 functions more consistently as a negative regulator of cell proliferation and inducer of apoptosis, through several

effectors (FIG. 2). Therefore, the main role of this protein is that of a sensor of acute or chronic alterations in normal cellular physiology and, more specifically, DNA and chromosomal integrity^{6,7}. Whereas initial analysis of *Trp53*-knockout mice suggested that this gene is dispensable for development¹³, subsequent studies pointed to its involvement in the differentiation of several tissues^{6-8,14-16}. Importantly, this molecule has also recently been implicated as a negative regulator of stem cell potential, with clear implications for cancer development¹⁷⁻¹⁹. p53 may carry out some of its functions in concert with other family members, such as p63 and p73, which are more closely connected with development. In fact, loss of p63 results in defective epithelial cell fate commitment, specifically in the skin and its adnexa^{20,21}, and loss of p73 affects neuronal development²². p73 is not discussed further, as only limited information is currently available regarding its possible connection with Notch²³. As a result of differential splicing, p63 is produced as several isoforms, and these can either counteract or increase the p53 effects by binding to common promoter sequences of target genes or through separate control of other targets²⁴⁻²⁹. Additionally, p53 itself can be differentially spliced to form several isoforms with potentially opposite effects³⁰. Although the best-studied mechanisms of p53 regulation are post-transcriptional³¹, a less appreciated but nevertheless important form of p53 regulation is at the level of *TP53* gene transcription³²⁻³⁷ (FIG. 2).

Control of p53 by Notch signalling

Notch signalling can either suppress or increase p53 activity in a context-dependent manner that is closely connected to tumour promotion or suppression. Four Notch receptors exist in mammalian cells (FIG. 1). Although most studies have so far focused on the interconnection between p53 and the NOTCH1 receptor, other Notch family members can also have important roles in carcinogenesis. For this reason, in the rest of this article I refer to Notch in general, rather than to NOTCH1, in the cases in which the specific contribution of the various family members is not known or in which NOTCH1 activity probably functionally overlaps with that of the other receptors.

Notch suppression of p53

The most convincing demonstration that increased Notch signalling promotes tumour formation, in both clinical and experimental settings, has been provided by studies on T cell acute lymphoblastic leukaemias (T-ALLs)^{10,11}. Most human T-ALLs harbour mutations of *NOTCH1*, which either facilitate protease-dependent activation of the receptor or increase the stability of its proteolytically cleaved activated form, the Notch intracellular domain³⁸. An elegant mouse model of the pathogenesis of the disease has been developed, based on a tetracycline-inducible activated *NOTCH1* transgene³⁹. In this model, Notch activation reduces p53 protein levels through increased activity of MDM2, an E3 ubiquitin ligase that targets p53 for degradation (FIG. 3a). The underlying mechanism involves decreased expression of ARF (encoded by *CDKN2A*), a key negative regulator of MDM2 activity. This may be a peculiarity of the system or also may apply to the clinical situation, in the fraction of human T-ALLs in which *CDKN2A* has not been deleted or mutated⁴⁰. Importantly, tumorigenesis in this system depends on sustained Notch activity, as attenuation of Notch expression dramatically increases p53 levels and tumour regression through apoptosis. In the

presence of activated NOTCH1 expression, increased p53 expression, either through MDM2 inhibition (by small-molecule MDM2 inhibitors such as Nutlin-3) or as a result of ionizing radiation, was sufficient for tumour regression³⁹.

Further studies support the hypothesis that suppression of p53 activity is a key aspect of Notch-induced tumorigenesis. In other work with human T-ALL, as well as breast cancer and keratinocyte cell lines, Notch activation was shown to increase cell survival through the activation of the PI3K–Akt pathway, which leads to increased MDM2 activity and consequent p53 degradation^{41–43} (FIG. 3a). A proposed underlying mechanism for Akt activation by Notch is the negative regulation of *PTEN* by HES1 (REF. 43), a canonical Notch effector that represses transcription³. In experimental settings, Notch activation was required for tumour formation in cells with wild-type p53 and *PTEN*, whereas loss of either protein rendered Notch activation dispensable^{44,45}. Similar conclusions have been drawn from clinical T-ALL studies, in which *PTEN* mutations leading to persistently increased Akt activity and decreased p53 levels can account for acquired resistance to Notch inhibitors⁴⁶.

p53 activity may also be suppressed by activated Notch through direct association⁴⁷. The amino terminus of activated NOTCH1 was reported to bind p53, resulting in the inhibition of p53 phosphorylation and DNA binding activity. A limitation of these potentially important studies is that they were carried out mostly with overexpressed proteins. The binding of endogenous p53 and activated NOTCH1 proteins was also detected, but at weak levels, pointing to the need for additional experimental approaches and validation in other cellular systems of direct and physiologically important Notch–p53 interactions.

In addition to T-ALL, other cases in which Notch activation has been implicated as a positive determinant of tumour formation include primary melanomas⁴⁸, breast cancer⁴⁹ and gliomas⁵⁰. Overall, the positive role of Notch in transformation has been linked to the induced expression of genes with oncogenic potential, such as *MYC*⁵¹ and *CCND1* (which encodes cyclin D1)⁵². However, upregulation of these genes can also lead to a replicative stress signal and consequent p53-dependent apoptosis¹⁰. Therefore, a second key function of Notch in transformation is to suppress apoptosis through downmodulation of p53 (REF. 10). It follows that increasing p53 activity using recently identified low-molecular-mass compounds that stabilize p53, such as Nutlin-3, or that induce refolding of p53 mutants into a form with wild-type activity, such as PRIMA-1 and RITA⁶, provides an attractive avenue for therapeutic intervention in Notch-induced tumorigenesis.

Notch activation of p53

In contrast to the above situations, p53 can be affected positively in systems in which Notch activation exerts a growth-inhibitory or pro-apoptotic function (FIG. 3b,c). In mouse and human keratinocytes, Notch signalling is essential to promote differentiation^{53–55} and suppress tumorigenesis^{56–59}. Cervical carcinoma is a clinically important type of keratinocyte-derived tumour that is causally linked to infection by high-risk human papillomaviruses (HPVs), such as HPV16 and HPV18 (REF. 60). A key determinant of HPV transformation is the E6 oncoprotein, which binds endogenous p53 and directs its degradation. Importantly, E6 is expressed at low levels in the early stages of HPV infection and in low-grade cervical intraepithelial neoplasias, when control of viral gene expression is

coupled with the keratinocyte growth and differentiation programme⁶⁰. E6 expression is then increased in high-grade malignant tumours⁶⁰.

Paralleling these findings, NOTCH1 expression is increased in early neoplastic lesions and well-differentiated parts of cervical cancer^{61–63}, and it is substantially downmodulated in the less differentiated and more aggressive parts of the tumours⁶⁴. This heterogeneous expression pattern suggests that NOTCH1 may have a permissive or tumour-promoting function in the early stages of cervical carcinogenesis, whereas it suppresses the later stages. In this context, both increased and decreased NOTCH1 function could lead to the suppression of p53 activity. In the early stages, in cells with limited HPV E6 expression, increased NOTCH1 levels could contribute to a decrease in p53 protein levels through the Akt and MDM2-dependent mechanism discussed above⁴². However, for the late stages of cervical carcinogenesis, downmodulation of NOTCH1 would probably be required. In fact, in cervical cancer cells in which endogenous NOTCH1 expression is down-modulated, increasing NOTCH1 activity causes growth arrest that is linked to down-modulation of E6 expression, through the inhibition of the activator protein 1 (AP1) transcription factor complex^{64–66} (FIG. 3b) and additional possible mechanisms^{65–67}. These may include CSL (also known as RBPJ κ), a DNA binding protein and a main mediator of the canonical Notch pathway that is converted by Notch from a repressor into an activator of transcription³ (FIG. 1). CSL was recently found to exert a negative regulatory function on *TP53* transcription in HeLa cells⁶⁸, which could be relieved by NOTCH1 activation.

Ewing's sarcoma is another type of human tumour that has recently been linked to suppression of Notch and p53 activity⁶⁹. These tumours, which arise in the bone and soft tissues of children and young adults, are characterized by a specific chromosomal translocation resulting in the fusion of the *EWSRI* (also known as *EWS*) gene to a gene encoding a member of the Ets family of transcription factors, most frequently friend leukaemia virus integration 1 (*FLII*)⁷⁰. Similarly to cervical carcinomas, most of these tumours retain wild-type p53, which, however, is poorly expressed. Persistent expression of *EWS-FLII* is required for tumour growth, and knockdown of *EWS-FLII* results in a marked increase of p53 expression and activity. Mechanistically, this has been linked to *EWS-FLII*-mediated suppression of Jagged 1 (JAG1) expression and consequent inhibition of Notch signalling⁶⁹. HEY1, which similarly to HES1 is a canonical Notch target and transcriptional repressor⁷¹, was upregulated as a consequence of *EWS-FLII* knockdown, inducing p53 activity through an indirect mechanism involving increased p53 protein without changes in mRNA levels⁶⁹ (FIG 3c).

A previous study had also indicated that p53 activity is increased by HEY1, as well as HES1, expression⁷². In this case, the underlying mechanism was attributed to HES1 or HEY1 repression of *MDM2* transcription, with a consequent increase of p53 protein levels and apoptosis (FIG. 3c). These findings have been validated in developmental systems *in vivo*, in which increased HES1 or HEY1 expression results in phenotypes similar to those caused by p53 overexpression⁷². They may also be relevant to cancer development, as Ras- and MYC-mediated transformation of mouse embryonic fibroblasts was abrogated by the expression of HEY1 in a p53-dependent manner⁷².

In keeping with the above findings and a previous report with NIH3T3 fibroblasts⁷³, increased NOTCH1 activity resulting from the genetic deletion of the FBXW7 (also known as SEL-10) ubiquitin ligase was also found to cause growth suppression in mouse embryonic fibroblasts⁷⁴. This was linked to p53 upregulation through an as yet undefined CSL-dependent mechanism.

Similar increases of p53 protein expression and activity have been connected with the induction of growth arrest and/or apoptosis by activated NOTCH1 expression in other tumour cell types, such as human hepatocellular⁷⁵ and oral (tongue)⁷⁶ carcinoma cells, as well as multipotent haematopoietic progenitor cells⁷⁷. p53 expression and activity also increase as a consequence of Notch activation in conditions not related to cancer in which activation of Notch signalling leads to apoptosis. These include early neural development⁷⁸, diabetic nephropathy and focal segmental glomerulosclerosis⁷⁹.

Overall, in contexts in which Notch signalling restricts growth and/or induces apoptosis, this has been frequently linked with positive regulation of p53 activity and expression. However, more complex situations may also exist. In fact, upregulation of p53 expression by Notch activation, at the level of gene transcription, has also been observed in glioma cells⁸⁰, but sustained Notch signalling in this case is important for survival⁵⁰. These apparently conflicting signals may be reconciled, at least in part, by the observation that Notch signalling in glioma cells is associated with the upregulation of the epidermal growth factor receptor (*EGFR*) gene⁸⁰, a cell survival gene reported to be a positive target of p53 (REFS 81,82).

Control of Notch by p53

Notch signalling can regulate p53 activity, but p53 can also regulate Notch, in reciprocal positive or negative feedback loops that are important for cell proliferation and cancer development.

p53 suppression of Notch

A main form of Notch regulation is at the level of Notch ligand expression and γ -secretase-dependent activation (FIG. 1). Expression of Jagged ligands can be under positive control of p53 (REFS 28,83), as discussed further below. However, expression of presenilin 1 (PSEN1), a key catalytic component of the γ -secretase protease complex, is under the transcriptional regulation of several pathways, including that of p53 (REF. 84). p53 can downregulate PSEN1 expression through either an indirect competitive mechanism with Ets transcription factors⁸⁵ or the induction of p21 (also known as p21^{WAF1/Cip1})^{86,87}, a negative regulator of transcription^{88,89}. Inhibition of PSEN1 expression by p53 and p21, with consequent downmodulation of Notch signalling, has been proposed as a mechanism for the increased death of neurons in p53-deficient mice⁸⁶, as well as the impaired maturation of thymocytes with p53 deletion⁹⁰. This mechanism may also be relevant to thymus cancer development and could account for the observed suppression of Notch activity by p53⁹⁰.

p53 induction of Notch

Another mechanism for the control of Notch activity by p53 is at the level of *NOTCH1* transcription. As mentioned above, in keratinocytes Notch signalling has an important pro-differentiation^{53–55} and tumour-suppressing function^{56–59}. Increased tumorigenesis resulting from Notch inhibition has been connected with the downmodulation of NOTCH1 expression in cutaneous squamous cell carcinomas⁵⁷ and basal cell carcinomas⁹¹. Downregulation of *NOTCH1* transcription in these tumours can be explained, at least in part, by compromised p53 function. In fact, in primary human keratinocytes, endogenous p53 binds to the *NOTCH1* promoter and p53 knockdown results in downmodulation of NOTCH1 expression, whereas increased p53 levels lead to NOTCH1 upregulation^{57,92,93}. Similarly, the already mentioned downregulation of NOTCH1 expression in HPV-positive cervical carcinoma cells can be explained by decreased p53 levels resulting from viral E6-dependent p53 degradation⁹³. Therefore, whereas NOTCH1 activation leads to the suppression of E6 expression and consequently to increased p53 levels, p53 in turn stimulates *NOTCH1* transcription, and this positive feedback loop is disrupted in malignant cervical carcinogenesis (FIG. 3b).

Control of NOTCH1 expression by p53 is also relevant for the keratinocyte response to ultraviolet (UV) light, a major aetiological agent of skin ageing and cancer^{94,95}. p53 has a key role in the DNA damage response of keratinocytes to UV light, controlling not only the decision between growth arrest and apoptosis, but also differentiation^{92,93,96}. Underlying differentiation is the induction of NOTCH1 expression through a p53-dependent mechanism, with NOTCH1 exerting a protective function against the DNA damage response to UVB light through suppression of forkhead box O3 (*FOXO3*), a key pro-apoptotic gene⁹².

In addition to keratinocytes and their malignant counterparts, NOTCH1 expression is under positive p53 control in lung and prostate cancer cells, in which growth suppression is also caused by increased Notch signalling⁹⁷, as well as in B cell chronic lymphocytic leukaemia cells, in which Notch signalling increases cell survival⁹⁸. In many cases, p53 was found to be specifically involved in the control of *NOTCH1* with little or no effects on other family members^{57,93,97}. A survey of the human genome by global chromatin immunoprecipitation⁹⁹, as well as direct chromatin immunoprecipitation assays⁵⁷, also pointed to the *NOTCH1* promoter as a direct p53 binding target in HCT116 colon carcinoma cells. Interestingly, however, p53 did not seem to affect NOTCH1 expression in these cells, pointing to a possibly cell type-specific mechanism of p53 control of NOTCH1 expression downstream of promoter recognition and binding⁵⁷.

Crosstalk between NotCH1 and p63

Whereas NOTCH1 activation restricts the growth potential of keratinocytes and promotes differentiation¹⁰⁰, p63 has been linked to epidermal cell fate determination and maintenance of self-renewing cell populations^{101,102}. p63 is expressed by keratinocytes of the proliferative compartment of the epidermis and initially committed cell populations, and its expression is suppressed with differentiation. Conversely, p63 is often upregulated in various epithelial tumours, including skin squamous cell carcinomas (for a review of this topic see REF. 103) in which Notch signalling is downmodulated⁵⁷. p63 upregulation in tumours has

been connected to antagonistic functions of p63 versus p53 and p73 on the control of downstream target genes involved in cell cycle withdrawal, apoptosis and cell senescence^{7,8,26,104}. As discussed below, reciprocal negative regulation has been established between NOTCH1 and p63 expression and activity, with important consequences for the balance between self-renewing and committed cell populations, as well as, possibly, carcinogenesis²⁴ (FIG. 4).

NOTCH1 control of p63

The molecular basis for the control of p63 expression in growing keratinocytes compared with differentiating keratinocytes, and in tumour cells, has been studied to a limited extent. NOTCH1 inhibits the expression of p63 in these cells both *in vitro* and *in vivo*²⁴. Negative regulation of p63 by NOTCH1 is probably cell type-specific (as the opposite effect was reported in NIH3T3 fibroblasts¹⁰⁵), is not an indirect consequence of growth arrest and is not caused by key mediators of NOTCH1 function in keratinocytes, such as members of the Hes and Herp families⁷¹ or p21 (REFS 55,88).

Transcription of *TP63* can be initiated at two different promoters, resulting in mRNAs for either the TA-p63 or N-p63 isoforms that contain or lack, respectively, an N-terminal transcription-activating (TA) domain²⁹. The major isoform expressed by keratinocytes is N-p63a. The promoter region for N-p63 contains multiple binding sites for interferon-responsive factors (IRFs) and nuclear factor- κ B (NF- κ B) subunits, with a potentially significant similarity to the interferon- β enhancer, which depends on a synergistic complex formation among these transcription factors¹⁰⁶. Both protein families may be involved in the control of p63 expression by NOTCH1.

Activation of the NOTCH1 pathway in keratinocytes results in the selective modulation of interferon-responsive genes, with some genes of this family being induced and others suppressed^{24,107}. Among the downmodulated genes are *IRF7*, a key regulator of the interferon-dependent transcription cascade with oncogenic potential^{108,109}, and *IRF3*, which physically interacts and functionally overlaps with IRF7 (REF. 110). Functionally, the ability of NOTCH1 activation to downmodulate p63 expression was overcome by IRF7 overexpression, and the combined knockdown of IRF3 and IRF7 resulted in decreased p63 expression²⁴. Importantly, the reduction of p63 levels by the concomitant IRF7 and IRF3 knockdown was less than that caused by activated NOTCH1 expression, suggesting that other mechanisms are also in place.

Biochemical and functional evidence indicates that p63 is also negatively regulated by NF- κ B in keratinocytes²⁴. As NF- κ B activity is induced in these cells with differentiation^{111,112}, as well as Notch activation⁵⁴, it is possible that suppression of p63 by Notch also involves NF- κ B activation. However, if NF- κ B is involved, Notch-dependent suppression occurs through a mechanism that is not blocked by expression of stabilized NF B inhibitor alpha (I Ba), which inhibits the canonical NF- κ B (p65 and p50) pathway²⁴.

An interesting possibility for future studies is whether the above mechanisms function in concert with the recently described microRNA-dependent regulation of p63

expression^{113,114}, given the already demonstrated interconnection between Notch and control of microRNA expression and function in other systems^{115,116}.

p63 control of Notch

p63 is regulated by Notch, but at the same time also controls the Notch pathway. As p53 and p63 share a common DNA recognition sequence²⁹, p63 may participate in the control of *NOTCH1* transcription. In fact, endogenous p63 can bind to the *NOTCH1* promoter, and there is defective NOTCH1 expression in the epidermis of mouse embryos with p63 deletion¹¹⁷. However, direct control of NOTCH1 expression by p63 may be limited to development, as in mature keratinocytes p63 functions as a selective modulator of downstream Notch signalling (see below) with little or no effect on *NOTCH1* transcription^{34,93}.

Persistently increased p63 expression counteracts the ability of Notch to restrict keratinocyte growth potential, through a mechanism involving suppression of p21 expression, as well as, possibly, upregulation of integrin receptors^{24,118} (FIG. 4). Concomitantly, p63 exerts both Notch-antagonistic and Notch-synergistic effects on differentiation. In fact, in parallel with a decreasing gradient of p63 expression from basal to upper epidermal layers, persistently increased expression of this protein suppresses differentiation markers of the granular and outermost layers, whereas it upregulates those of early entry into differentiation (spinous layer), which are also under positive Notch control^{24,119}.

Both intracellular and intercellular regulatory mechanisms are probably involved in this dual role of p63 in differentiation. Intracellularly, p63 functions as a selective modulator of NOTCH1-dependent transcription, with *HES1* as one of its direct negative targets²⁴. *HES1* in turn is a modulator of differentiation^{24,34,120}.

Besides impinging on intracellular Notch signalling mechanisms, p63 has the potential to induce activation of this pathway in neighbouring cells. *JAG1* and *JAG2* transcription is positively regulated by p63 (REFS 28,83). Induction of these genes by p63 can be important for normal skin homeostasis, serving as a positive reinforcement signal to synchronize differentiation of keratinocytes in the suprabasal epidermal layers (FIG. 4). This function of p63 in inter-cellular communication may extend to the thymus, where similar defects in $\gamma\delta$ T cell maturation have been observed in *Trp63*^{-/-} and *Jag2*^{-/-} mice, and the *Trp63*^{-/-} phenotype can be explained by decreased *JAG2* expression by thymic epithelial cells¹²¹.

Crosstalk by downstream mediators

Cross-regulation between the Notch and p53 pathways may also occur further downstream, at the level of shared intermediates. The Notch transcriptional co-activator mastermind-like 1 (MAML1), or related family members, is required for increased levels of Notch-CSL-dependent transcription, as MAML1 recruits histone acetyl-transferases, such as p300, to the Notch-CSL complex¹²²⁻¹²⁷. Although initially identified as specific mediators of Notch signalling, MAML proteins have now been implicated in other signalling pathways, including those of muscle cell differentiation, β -catenin and p53 (REF. 128). More specifically, a direct MAML1-p53 association has also been described¹²⁹. The N-terminal

region of MAML1 binds both Notch and p53, raising the possibility of direct competition for this binding site¹²⁹. A more indirect mechanism is also possible. The N-terminal region of MAML1 also binds to p300 (REF. 130), and competition for p300 binding has previously been implicated in the suppression of Notch–MAML1-dependent transcription by p53 (REF. 124).

Another possible connection between Notch and p53 is provided by NUMB, an endocytic protein and determinant of asymmetric cell division that is implicated in the negative control of Notch function³. NUMB was found to bind MDM2 in a yeast two-hybrid assay and was subject to MDM2-dependent degradation¹³¹, suggesting that MDM2 can increase Notch signalling through NUMB downmodulation. By contrast, NUMB itself has recently been found in a complex with MDM2 and p53, in which it prevented p53 ubiquitylation and degradation¹³². Therefore, it has been proposed that the frequent loss of NUMB expression in breast cancer contributes to an aggressive tumour phenotype not only by upregulating a Notch-positive growth signal but also by decreasing p53 activity¹³².

Cross-interacting pathways

In keratinocytes, in addition to increasing proliferation, EGFR signalling suppresses differentiation¹³³. This may be relevant to the balance between self-renewing and committed keratinocytes in the basal proliferative compartment of the epidermis, where EGFR is expressed, as well as in squamous cell carcinomas, in which EGFR signalling is characteristically increased (for a review of this topic see REF. 134). In a chemical genetics screen for small-molecule activators of Notch signalling, EGFR signalling through Erk was identified as a key negative regulator of NOTCH1 expression in keratinocytes, with downregulation of *TP53* — through AP1 — as an underlying mechanism³⁴. This concomitant mode of regulation of p53 and NOTCH1 expression by EGFR signalling was validated in organ cultures of intact human skin and cutaneous squamous cell carcinomas, as well as a mouse model of EGFR-dependent skin cancer. EGFR has become an important therapeutic target in cancer, and several selective EGFR inhibitors have now been approved for clinical use. Suppression of Notch signalling in cancer cells can synergize with these compounds in the induction of apoptosis³⁴ (FIG. 5). Therefore, Notch inhibitors may increase the effects of EGFR-inhibitory agents on tumours, while simultaneously ameliorating their well-known toxic effects on the skin, which have been attributed, at least in part, to aberrant differentiation¹³⁵. Similar synergistic induction of apoptosis has been observed in other cancer cells following concomitant treatment with Notch inhibitors and either ERBB2-specific antibodies¹³⁶, p53 inducers⁹⁸ or genotoxic agents⁹⁷.

Transforming growth factor- β (TGF β)–Smad signalling can be another important point of convergence of the p53 and Notch pathways. In fact, a close connection between p53 and TGF β –Smad function has been established¹³⁷, and Smad proteins also functionally and biochemically interact with activated NOTCH1 (REFS 138–140). This is relevant for the tumour-suppressing function that NOTCH1 and p53 share in specific settings, such as in keratinocytes, with p21 as an important common downstream target¹⁴¹. NF- κ B signalling can also be involved in the reciprocal modulation of Notch and p53 signalling. A reciprocal crosstalk between p53 and NF- κ B has been established¹⁴², and NF- κ B may be implicated in

the positive control of p53 expression¹⁴³ and ubiquitin-dependent p63 degradation¹⁴⁴. NF- κ B can also positively control Notch ligand expression¹⁴⁵, itself subject to either positive or negative Notch control (for a review of this topic see REF. 146).

Future directions

Recent debate on the tumorigenic potential of distinct cancer cell populations^{147–149} has focused on the notion of ‘stemness’ as a dynamic rather than an intrinsically fixed property of cells^{150–152}. A reversible equilibrium probably exists between cells with high self-renewal potential and more committed cell populations, with reprogramming of individual cells associated with stochastic fluctuations of the transcriptome¹⁵³. An exciting area for future studies is the effect that the interconnection between Notch and p53 or p63 can have on self-renewal of cells compared with commitment to senescence or differentiation. As discussed at the beginning of this article, in situations in which Notch has a tumour-promoting function, loss or down-modulation of p53 activity renders Notch activation dispensable^{44–46}. Importantly, however, in many tumours *TP53* mutations do not abrogate p53 function but confer new, or a wider range of, functions on p53 (REF. 6) that may considerably affect the crosstalk with Notch.

In addition to intrinsic control mechanisms, self-renewal of cells is dependent on cues from the environment. In multicellular organisms, cells function as integrated networks, with intrinsically heterogeneous behaviour of individual cells¹⁵⁴ being synchronized by multiple forms of homotypic and heterotypic intercellular communication, communication at a distance (as mediated, for instance, by hormonal influences) and communication in response to inputs from the external environment. Crosstalk between the Notch pathway and p53 and p63 pathways in these contexts remains to be explored.

Finally, the design of new or improved therapeutic approaches to complex diseases, such as cancer, is dependent on an understanding of the functional integration of cell and tissue homeostasis temporally and spatially. Oscillations of p53 and MDM2 have emerged as a key component of cell cycle progression and DNA repair checkpoint controls¹⁵⁵. Intrinsic oscillation mechanisms of Notch signalling have also been demonstrated to be connected with development^{156,157}. Therefore, an important possibility is that the Notch pathway and the p53 and p63 pathways are also dynamically integrated, yielding cell state fluctuations that differ among various cell types, and in normal cells compared with cancer cells. Selective targeting of these pathways in specific tissues and at various times could greatly improve the efficacy of new cancer therapy approaches and reduce their toxic side effects.

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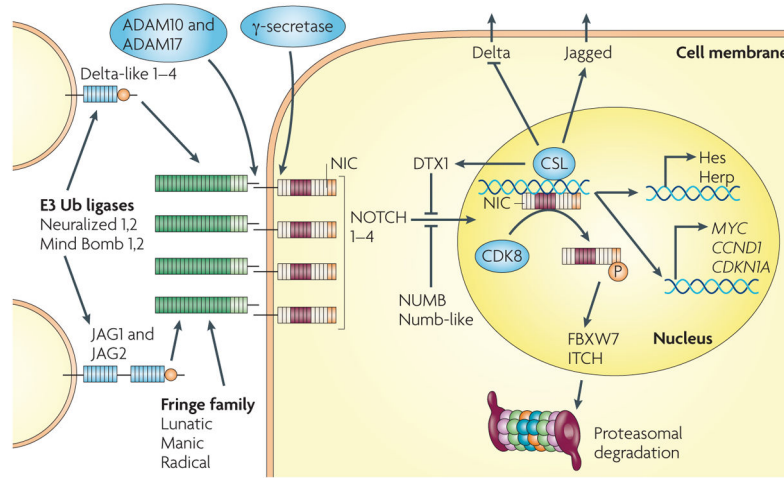


Figure 1. The notch signalling module

Two families of transmembrane Notch ligands and four Notch receptors exist in mammalian cells. The ligands must be ubiquitylated by specific e3 ubiquitin (Ub) ligases to induce Notch activity, which they have been proposed to do through several mechanisms, such as exerting a pulling force on the engaged receptor, ligand clustering or internalizing, and undergoing modification and subsequent reinsertion into the membrane. Modification of the extracellular domain of Notch receptors by Fringe family glycosyltransferases allows differential activation by the Delta or Jagged ligands. Following ligand engagement, Notch receptors are cleaved first by an ADAM metalloproteinase and then, at the juxta-membrane region, by the presenilin- γ -secretase complex. The resulting Notch intracellular domain (Nic) translocates to the nucleus, where it associates with the DNA binding protein CSL (also known as rBPJ), converting it from a repressor to an activator of transcription. This induces the transcription of two classes of target genes. Genes of the Hes family, which encode basic helix-loop-helix transcriptional repressors, and genes of the Herp family, which encode related but distinct basic helix-loop-helix proteins that heterodimerize and cooperate with Hes family members, are ubiquitously induced. Cell cycle regulatory genes, such as *MYC*, *CCND1* (which encodes cyclin D1)⁵² and *CDKN1A* (which encodes p21) are induced in a cell type-specific manner. In parallel, Notch activation controls neighbouring cells, through an opposite modulation of ligands of the Delta and Jagged families, which can provide a basis for lateral inhibition or lateral induction, respectively (as described in the main text). The Notch transcription-activating complex is terminated by phosphorylation of activated nuclear Notch by cyclin-dependent kinase 8 (cDK8), with subsequent recognition by the ubiquitin ligase FBXW7 (also known as seL-10) and proteasome-dependent degradation. Negative regulatory mechanisms are also provided by the e3 ubiquitin ligases Deltex 1 (DTX1) and iTch and by NUMB, an asymmetrically distributed protein that has been linked to the recruitment of Notch into endocytic vesicles but also to NOTCH ubiquitylation. For more detailed information, see REFS 3,4,146.

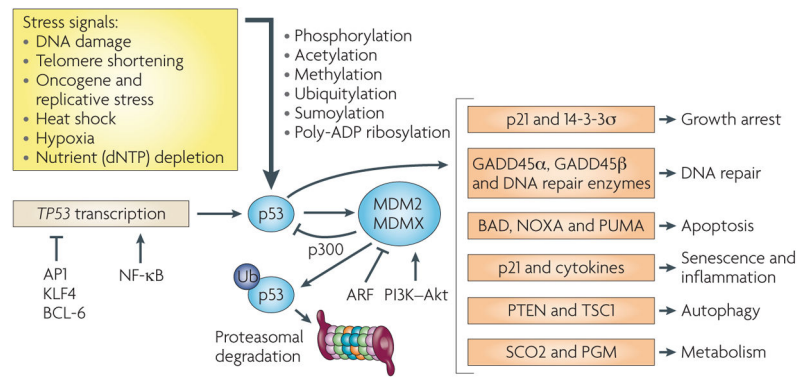


Figure 2. The p53 signalling module

p53 activity is induced by many stress-related signals and is under the control of various transcriptional and post-transcriptional regulatory mechanisms. These include acetylation, methylation, phosphorylation and poly-ADP ribosylation, which have been implicated in the control of p53 nuclear versus cytoplasmic localization, as well as gene target selectivity. Phosphorylation of p53 at specific serine and threonine residues affects recognition by the ubiquitin (Ub) E3 ligase MDM2 and the related protein MDMX (also known as MDM4). Although the main function of MDM2 is to decrease p53 stability, MDM2 and MDMX can also suppress p53 transcriptional activity by competing with the acetyltransferase p300 for p53 binding. The role of p53 phosphorylation *in vivo* remains to be established, but the negative regulatory function of MDM2 and MDMX has been confirmed by mouse genetic models and by the use of small molecules, such as Nutlin-3, that block p53–MDM2 interactions. As *MDM2* is one of the direct transcriptional targets of p53, the short and long arms of the negative feedback loop resulting from p53-dependent induction of *MDM2* and MDM2-dependent suppression of p53 activity provide the basis for the oscillatory behaviour of the p53 pathway in response to DNA damage¹⁵⁵. For more detailed information on the p53 pathway, as well as the consequences of *TP53* mutations not covered in this article, see REFS 6–8,31. AP1, activator protein 1; BAD, Bcl-2-associated agonist of cell death; GADD45a, growth arrest and DNA-damage-inducible-a; KLF4, Kruppel-like factor 4; NF-κB, nuclear factor-κB; PGM, phosphoglycerate mutase; scO2, synthesis of cytochrome *c* oxidase 2; Tsc1, tuberous sclerosis 1.

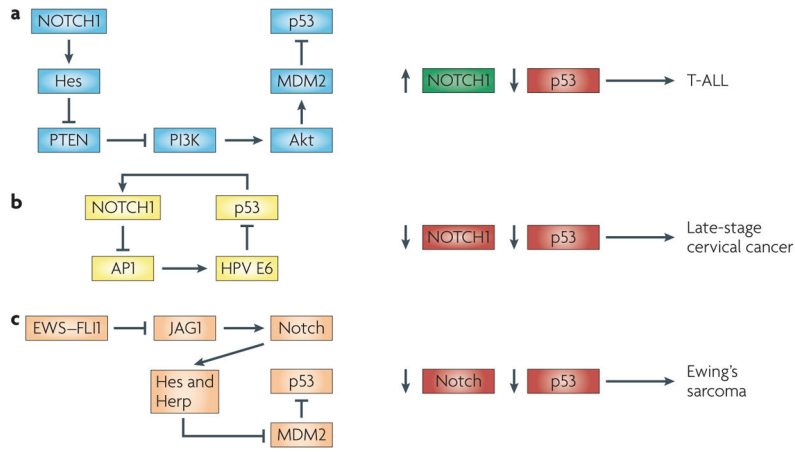


Figure 3. notch and p53 control

a | The oncogenic function of Notch signalling in T cell acute lymphoblastic leukaemia (T-ALL) is connected with the downmodulation of p53 through increased MDM2 activity. As discussed in the main text, a proposed mechanism, based on experimental and clinical studies^{43–46}, involves activation of the Pi3K–Akt pathway through Hes family-mediated down-modulation of PTeN expression. **b** | A positive feedback loop between p53 and NOTcH1 can counteract cervical carcinogenesis. Advanced stages of cervical carcinogenesis are linked to a concomitant upregulation of the human papillomavirus (HPv) e6 oncoprotein and downmodulation of *NOTCH1*. p53 protein levels and activity are increased by NOTcH1 activation in cervical carcinoma cells through the suppression of activator protein 1 (AP1)-dependent transcription of HPV e6. In turn, *NOTCH1* is a direct positive target and is induced by increased p53. **c** | In ewing’s sarcoma, the *EWSR1*–friend leukaemia virus integration 1 (*FLI1*) translocation product (eWs–FLi1) is a negative regulator of Jagged1 (JAG1) expression, with consequent suppression of Notch activity. The Notch pathway in this system positively regulates p53 activity through a mechanism involving transcriptional repression of *MDM2* by Hes and Herp family transcriptional repressors.

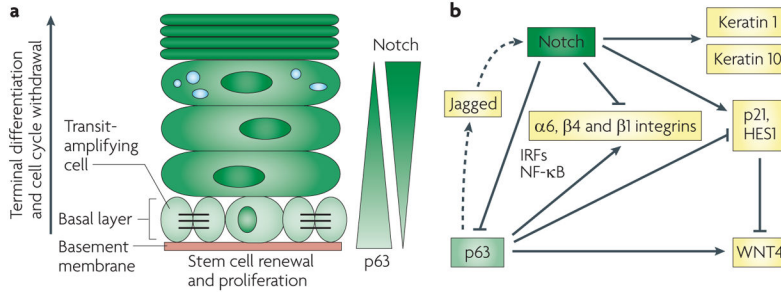


Figure 4. Crosstalk between p63 and Notch

reciprocal feedback and feedforward loops between Notch and p63 contribute to the balance between self-renewing keratinocyte populations and cells at various stages of commitment to differentiation. **a** | in the interfollicular epidermis, keratinocytes undergo a vertical programme of differentiation, as they proceed from the basal proliferating compartment to the upper terminally differentiating layers. in the proliferative compartment, a dynamic equilibrium probably exists between a minor fraction of slow cycling cells with increased self-renewal potential (putative stem cells) and already-committed populations that are actively dividing but only for a limited number of times (transit-amplifying cells). Opposing gradients of p63 expression and Notch activity exist in the basal but not the upper epidermal layers. This may result, in part, from their reciprocal negative regulation. **b** | Mechanistically, increased Notch activity suppresses the expression of p63 through modulation of interferon-responsive factors (irFs) and nuclear factor- κ B (NF- κ B) signalling and, possibly, microRNAs. in turn, p63 binds to the *HES1*, *CDKN1A* (which encodes p21) and *WNT4* promoters, counteracting the Notch effects on their expression (*WNT4* is suppressed by Notch activation through an indirect mechanism mediated by both Hes1 and p21). p63 and Notch also increase and suppress, respectively, integrin expression (α 6, β 4 and β 1 integrins), through less well-defined mechanisms. in parallel with these antagonistic effects, p63 can also synergize with Notch signalling in the early steps of differentiation (characterized by keratin 1 and keratin 10 expression), through a paracrine mechanism (illustrated by dotted arrows) involving the induction of expression of Jagged ligands and Notch activation in neighbouring cells. Downmodulation of p63 by increased Notch signalling could then be a signal for later differentiation stages to occur.

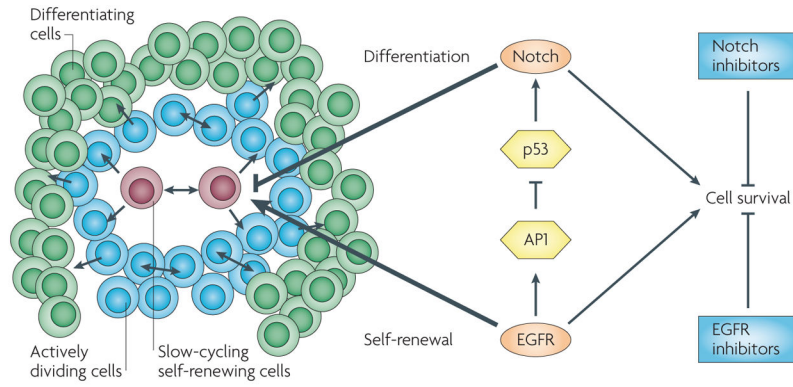


Figure 5. eGFr–p53–notch: an integrated switch between tumour cell differentiation and apoptosis

As in normal keratinocytes, in skin squamous cell carcinomas (illustrated on the left of the figure as an agglomerate of cells at various stages of the differentiation process) epidermal growth factor receptor (eGFr) signalling downmodulates NOTch1 expression through activator protein 1 (AP1)-dependent suppression of *TP53* transcription. squamous cell carcinomas are intrinsically heterogeneous and contain cells that have increased self-renewing potential but are possibly slow-cycling, actively dividing cells and cells that undergo differentiation. eGFr promotes self-renewal by increasing the proliferation or survival of both slow-cycling and actively dividing cells. conversely, Notch restricts proliferation potential and promotes differentiation. Although eGFr and Notch can exert opposite effects on differentiation, they both increase cell survival. As a result, pharmacological inhibition of both pathways can synergize in the induction of apoptosis.

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