Commentary

Cooperation between Wnt and Notch signalling in human breast cancer

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Abstract

The Wnt and Notch signalling pathways play major roles in mammary gland development and tumourigenesis. During development, these pathways have opposing effects. However, in a recent paper Ayyanan and coworkers show that expression of Wnt1 is sufficient to transform primary human mammary epithelial cells, and that this is in part due to activation of the Notch pathway. This indicates that during tumourigenesis the two pathways cooperate. Here we ask why activation of Wnt signalling alone is sufficient to cause transformation; whether there is evidence for inhibitory crosstalk between the pathways during tumourigenesis; and whether cooperation between these pathways occurs in other forms of cancer.

Through retroviral expression of Wnt1, Ayyannan and coworkers were able to demonstrate that a subset of primary human mammary epithelial cells (HMECs) display increased proliferation, an ability to form multicellular spheres and a failure to senesce following exposure to Wnt1 for a period of several weeks [1]. Furthermore, these cells formed tumours when they were injected into immunocompromised mice. This is rather unusual because expression of more than one oncogene is normally required to transform epithelial cells. For example, human embryonic kidney cells cannot be transformed by expression of hTERT (the catalytic subunit of telomerase), SV40 large T antigen, or H-ras alone [2]. In fact, it requires the expression of all three because several different cellular properties must be derailed at once, including cell proliferation, apoptosis, senescence, adhesion, polarity and growth.

Consequently, we ask is there evidence that Wnt signalling can regulate all of these cellular properties simultaneously? Wnt signalling is known to affect cell proliferation and growth in colorectal cancer by stimulating expression of *CyclinD1* and *c-myc*, respectively [3]. Ayyannan and coworkers also provide evidence that telomerase activity was activated, as telomere length was maintained preventing senescence, and that the p16/Rb and p53 checkpoints were disrupted affecting the control of both proliferation and apoptosis [1]. In the latter case, this evidence stems from biochemical data showing that Wnt1 expression in HMECs elicits a DNA damage response. In normal cells, this would cause cell arrest at the p16/Rb or p53 checkpoint. The continued proliferation of Wnt1-expressing HMECs, despite high levels of p53, indicates that these checkpoints have been inactivated or that cells with a nonfunctional form of p53 have been selected. This can also explain the observed abnormal karyotypes. However, Wnt/β-catenin signalling can stimulate chromosomal instability in its own right in colorectal cell lines [4]. In addition to these effects, local Wnt signalling may regulate apical-basal cell polarity by depleting Lethal giant larvae and, hence, promoting the accumulation of the apical partioning-defective 3/partitioning-defective 6/atypical protein kinase C complex within a specific region of the cell [5]. In contrast, global Wnt signalling will deplete Lethal giant larvae within the whole cell, preventing polarization. Therefore, constitutive Wnt signalling may contribute to the disruption of apical-basal cell polarity seen during the transition from hyperplastic lesions to benign disease [6].

Lastly, Wnt signalling stimulates the self-renewal of many different adult stem cell populations [7]. Therefore, the expression of Wnt1 in HMECs may increase the number of stem cells within this cell population; interestingly, stem cells exhibit resistance to apoptosis and failure to senesce similar to those in transformed cells. The increased number of cells expressing stem/progenitor cell markers in tumours from mouse mammary tumour virus-Wnt1 mice suggests that this is likely [8]. Furthermore, given the possible role of cancer stem cells in tumour development, this regulation of stem cell self-renewal may contribute significantly to the Wnt1-mediated transformation of HMECs. Altogether, it is clear that Wnt signalling regulates many cellular properties at once, unlike classical oncogenes, which may explain its ability to

HMEC = human mammary epithelial cell.

transform HMECs alone. This also illustrates how developmental signalling pathways can deregulate cell behaviour during the early stages of cancer.

Ayyanan and coworkers [1] also demonstrated that expression of Wnt1 in HMECs led to subsequent activation of Notch signalling. Furthermore, studies of a panel of 34 breast carcinomas showed concomitant upregulation of the Wnt target genes *Lef1* and *Axin2* along with *Delta-like 3* and *Delta-like 4*, suggesting that the same process takes place in tumours. Expression of function-blocking Notch ligands abrogated HMEC transformation by Wnt1, demonstrating that there is a need for Notch signalling. However, activation of Notch signalling alone was not sufficient to cause transformation. What, then, is the role of Notch signalling in Wnt-mediated trasformation? One possibility is the upregulation of the transcriptional repressor Slug [9], which can both stimulate an epithelial to mesenchymal transition, and prevent *PUMA* expression, a pro-apoptotic member of the *Bcl-2* family, in response to p53 activation [10] In fact, Notch signalling has been shown to regulate apoptosis through many different mechanisms, including inhibition of p53 [11], which probably reflects regulation of key survival pathways within cells such as the Akt pathway. Notch signalling can also stimulate cell proliferation in the rat kidney cell line RKE by increasing *cyclinD1* expression [12], and it may affect cell growth through regulation of *c-myc* expression [13]. Finally, like the Wnt pathway, Notch signalling has been shown to stimulate the self renewal of stem cells, including HMECs [14,15].

Since Notch signalling can regulate many of the same cellular properties as the Wnt pathway, this raises the question of why is Notch signalling alone insufficient to cause HMEC transformation? One possibility is a failure to activate telomerase activity, which thus far is not known to be affected by Notch signalling. An alternative is expression of the cyclin-dependent kinase inhibitor p21, preventing cell proliferation, which has been shown to be a direct target of the pathway in keratinocytes [16] and is activated in the normal breast epithelial cell line MCF 10A in response to Notch signalling (Stylianou S, Brennan K, unpublished data). Interestingly, p21 levels do not rise in HMECs following Wnt1 expression, even though Notch signalling is activated. One possibility is the induction of c-Myc expression by Wnt signalling, which in turn downregulates p21 expression [17]. Alternatively it may reflect modulation of Notch signalling by the Wnt pathway. Inhibitory crosstalk has been well documented in these pathways between Dishevelled and Notch, Notch and β-catenin, and glycogen synthase kinase-3β and Notch [18,19]. Furthermore, inhibitory crosstalk between the pathways has been reported in mammary gland cells. The pathways have opposing effects on the morphogenesis of the TAC2 mouse MEC cell line; Wnt signalling promotes branching of the epithelial ducts formed by TAC2 cells, whereas activation of the Notch pathway blocks this morphological differentiation [20]. However, when both pathways are activated concomitantly, the TAC2 cells still form branched ducts, indicating that Wnt signalling, in this instance, can modulate Notch signalling.

Finally, are there other instances in which Wnt and Notch signalling are closely linked during tumour development? In fact, examples can be found in tissues derived from all three germ layers. Within the colon, the two pathways tightly control the development and maintenance of the colonic epithelium [15]. Activation of the two pathways together promotes stem cell renewal, whereas activation of Wnt and Notch signalling separately promotes differentiation into secretory cells and enterocytes, respectively. Furthermore, the same activation of the Notch pathways in response to Wnt signalling is seen in colorectal tumours [21]. However, it is not currently clear whether Notch signalling is required for Wnt-mediated transformation of the colonic epithelium. Similarly, signalling through both pathways is required for the maintenance of haematopoietic stem cells and, again, increased Wnt signalling leads to activation of the Notch pathway [22]. Furthermore, both pathways are dysregulated in leukaemia [23], although there has been little study of whether crosstalk occurs between the two pathways.

Wnt and Notch signals do not always cooperate in tumour formation. Within the skin, both pathways are again involved in stem cell regulation and differentiation. However, they have opposing effects, with Notch signalling promoting differentiation and Wnt signalling promoting stem cell self renewal and proliferation [24]. Furthermore, *Notch1* has been shown to act as a tumour suppressor gene in the epidermis [25], whereas Wnt signalling is clearly oncogenic [7,26].

To conclude, it is now clear that signalling pathways such as the Wnt and Notch pathways, which regulate development and tissue maintenance in the adult, control cell fate decisions by manipulating cell proliferation, death, polarity, senescence and adhesion, as well as the expression of cell type specific proteins and transcription factors. Consequently, it is not surprising that these pathways play a profound role in cancer. In addition, misregulation of these pathways is often associated with early stages of tumourigenesis. Finally, it is clear that the Wnt and Notch pathways are intimately intertwined in both stem cell self-renewal and cancer.

Competing interests

The authors declare that they have no competing interests.

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