Abstract

The Wnt and Notch signalling pathways play major roles in mammary gland development and tumorigenesis. 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 partitioning-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...
transform HMECs alone. This also illustrates how develop-
mental 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 Left1 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 transformation? 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
germs 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. Conse-
quentially, 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.

Acknowledgements
The authors’ research is supported by grants from the BBSRC and
Breast Cancer Campaign

References
1. Ayyanan A, Civenni G, Ciarloni L, Morel C, Mueller N, Lefort K,
Mandinova A, Raffoul W, Fiche M, Dotto GP, et al.: Increased
Wnt signaling triggers oncogenic conversion of human breast epithelial cells by a Notch-dependent mechanism. Proc Natl Acad Sci USA 2006, 103:3799-3804.

2. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA: Creation of human tumor cells with defined genetic elements. Nature 1999, 400:464-468.

3. Morin PJ: beta-catenin signaling and cancer. Bioessays 1999, 21:1021-1030.

4. Itoh K, Aoki M, Sugai M, Harada N, Miyoshi H, Tsukamoto T, Mizoshita T, Tatamatsu S, Seno H, Chiba T, et al.: Chromosomal instability by beta-catenin/TCF transcription in APC or beta-catenin mutant cells. Oncogene 2006 [Epub ahead of print].

5. Dollar GL, Weber U, Modzik M, Sokol SY: Regulation of Lethal/Notch inbreeding in Drosophila. Nature 2004, 437:1376-1380.

6. Aranda V, Haire T, Nolan ME, Calarco JP, Rosenberg AZ, Fawcett JP, Pasonow T, Muthuswamy SK: Par6-aPKC uncouples ErbB2 induced disruption of polarized epithelial organization from proliferation control. Nat Cell Biol 2006, 8:1235-1245.

7. Reya T, Clevers H: Wnt signaling in stem cells and cancer. Nature 2005, 434:843-850.

8. Liu S, Dountu G, Wicha MS: Mammary stem cells, self-renewal pathways, and carcinogenesis. Breast Cancer Res 2005, 7:86-96.

9. Timmerman LA, Grego-Bessa J, Raya A, Bertran E, Perez-Pomares JM, Diez J, Aranda S, Palomo S, McCormick F, Izpisua-Belmonte JC, et al.: Notch promotes epithelial-mesenchymal transition during cardiac development and oncogenic transformation. Genes Dev 2004, 18:99-115.

10. Wu WS, Heinrichs S, Xu D, Garrison SP, Zambetti GP, Adams JM, Look AT: Slug antagonizes p53-mediated apoptosis of hematopoietic progenitors by repressing puma. Cell 2005, 123:641-653.

11. Leong KG, Karsan A: Recent insights into the role of Notch signaling in tumorigenesis. Blood 2006, 107:2223-2233.

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

13. Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Look AT, DasGupta R: Notch1 functions as a tumor suppressor in mouse skin. Nat Genet 2005, 37:1376-1380.

14. Donut G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS: Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res 2004, 6:R605-R615.

15. Cronier C, Stamatski D, Lewis J: Organizing cell renewal in the intestine: stem cells, signals and combinatorial control. Nat Rev Genet 2006, 7:349-359.

16. Rangarajan A, Talora C, Okyama R, Nicolas M, Mammucari C, Oh H, Aker JC, Krishna S, Metzger D, Chambon P, et al.: Notch signaling is a direct determinant of keratinocyte growth arrest and entry into differentiation. EMBO J 2000, 20:3427-3436.

17. van de Wetering M, Sancho E, Verweij C, de Loo W, Oving I, Hurlstone A, van der Horn K, Batlle E, Clevers H, Dotto GP, Adams AP, et al.: The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. Cell 2002, 111:241-250.

18. Hayward P, Brennan K, Sanders P, Balay O, DasGupta R, Perri- mon N, Martinez Arias A: Notch modulates Wnt signalling by associating with Armadillo/beta-catenin and regulating its transcriptional activity. Development 2005, 132:1819-1830.

19. Martinez Arias A, Zecchin V, Brennan K: CSL-independent Notch signaling: a checkpoint in cell fate decisions during development? Curr Opin Genet Dev 2002, 12:524-533.

20. Uyttendaele H, Soriano J, Montesano R, Kitajewski J: Notch4 and Wnt-1 proteins function to regulate branching morphogenesis of mammary epithelial cells in an opposing fashion. Dev Biol 1998, 196:204-217.

21. van Es JH, van Ginneken ME, Riccio O, van den Born M, Vooijs M, Berghel H, Goezimn M, Robine S, Winton DJ, Radtke F, et al.: Notch/gamma-secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. Nature 2005, 435:959-963.

22. Duncan AW, Rattis FM, DiMascio LN, Congdon KL, Pazianos G, Zhao C, Yoon K, Cook JM, Willert K, Gaiano N, et al.: Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. Nat Immunol 2005, 6:314-322.

23. Weerlampati K, van Dongen JJ, Staal FJ: Notch and Wnt signaling in T-lymphocyte development and acute lymphoblastic leukemia. Leukemia 2006, 20:1197-1205.

24. Lowell S, Jones P, Le Roux I, Dunne J, Watt FM: Stimulation of human epidermal differentiation by delta-notch signaling at the boundaries of stem-cell clusters. Curr Biol 2000, 10:491-495.

25. Nicolas M, Wolf A, Raj K, Kummer JA, Mill P, van Noort M, Hui CC, Clevers H, Dotto GP, Radtke F: Notch1 functions as a tumor suppressor in mouse skin. Nat Genet 2003, 33:416-421.

26. Bhatia N, Spiegelman VS: Activation of Wnt/beta-catenin/TCF signaling in mouse skin carcinogenesis. Mol Carcinog 2005, 42:213-221.