

Commentary

Wnt signalling via the epidermal growth factor receptor: a role in breast cancer?

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Abstract

Recent data have suggested the epidermal-growth-factor receptor (EGFR) as a point of convergence for several different classes of receptor. Civenni and colleagues have now demonstrated crosstalk between Wnt signalling and the EGFR, showing that in breast epithelial cells Wnts activate downstream targets of the EGFR, including cyclin D1. Given the role of members of these pathways in the aetiology of breast cancer and as markers of outcome and potential therapeutic targets in breast cancer, this observation has a number of potential implications important for both the basic biology of breast cancer and the clinical management of the disease.

Keywords: breast cancer, cyclin D1, epidermal-growth-factor receptor (erbB1), Wnt pathway

Introduction

The Wnt family of secreted growth factors plays key roles in directing cell patterning both during development and in adult tissues. Consequently, it is not surprising that signalling pathways downstream of Wnts have been implicated in oncogenesis. While Wnts can also signal through other pathways, to date the majority of investigation into the potential role of Wnt signalling in the development of cancer has focused on the Wnt/ β -catenin pathway [1,2]. In this pathway, Wnts activate β -catenin signalling and consequently modulate the expression of specific target genes that regulate cell proliferation, apoptosis, and cell fate [1,2]. The recent publication by Civenni and colleagues [3] demonstrating that Wnt overexpression in mammary epithelial cells also activates signalling via the epidermal-growth-factor receptor (EGFR; erbB1) points to a further potential mechanism that may contribute to the oncogenic potential of Wnts in the mammary gland.

Wnt signalling and mammary oncogenesis

Several Wnts are regulated during mammary development [4] and Wnt-4 has been identified as a progesterone

target essential for lobuloalveolar development during pregnancy [5]. Wnt-1 and -3 were first identified as potential mammary oncogenes because of their frequent activation by insertion of the mouse mammary tumour virus (MMTV) [6,7]. Subsequent experiments showed that Wnt-1 and Wnt-3A, but not all Wnt proteins, were highly transforming for mammary epithelial cells *in vitro* [8], and that Wnt-1 is overexpressed in breast cancer [9]. Several oncogenic events that cooperate with Wnt overexpression in experimental models of mammary oncogenesis have been identified, including inactivation of p53 and activation of various fibroblast growth factor genes [10]. Evidence suggesting that Wnt signalling through β -catenin is the primary pathway for Wnt-mediated oncogenesis in the mammary gland includes the similarities between the effects of overexpression of activated β -catenin and Wnts [11,12], although additional effectors are also likely to be involved [12]. The downstream effectors of β -catenin include the cell cycle regulatory molecules c-Myc and cyclin D1, both well known mammary oncogenes that are overexpressed in Wnt- or β -catenin-induced mouse mammary tumours [11,12].

Crosstalk between Wnt and erbB signalling

Links between the erbB family of receptor tyrosine kinases and Wnt signalling were suggested by the observation that Wnt-1 and Wnt-3 were commonly overexpressed when the latency of mammary tumours induced by overexpression of the EGFR ligand TGF- α was reduced by infection with MMTV [13]. Subsequent work indicated that this could be mediated, at least in part, by direct interaction between β -catenin and EGFR/erbB2 heterodimers [14]. The identification by Civenni and colleagues [3] of a further mechanism linking these two key signalling pathways has potentially major implications because of evidence suggesting they are involved in the aetiology of breast cancer. For example, the EGFR is overexpressed in a significant fraction of breast cancers and appears to confer a poor prognosis, at least in the subgroup of patients that are negative for estrogen receptor [15].

Overexpression of Wnt-1 and Wnt-5a in HC11 mammary epithelial cells or treatment with conditioned medium from cells overexpressing these Wnts activated β -catenin, as expected [3]. Surprisingly, however, these treatments also stimulated EGFR tyrosine phosphorylation and activation of extracellular signal-regulated kinase (ERK)1/2 activation, events more usually associated with treatment with erbB ligands [3]. Inhibition of EGFR kinase activity or addition of secreted Frizzled-related protein-1 (sFRP-1), which competes with Wnts for Frizzled receptors, both prevented this effect; this observation is consistent with the conclusion that Wnt-1 and Wnt-5A activated mitogen-activated protein kinase (MAPK) signalling by EGFR. TGF- α and other EGFR ligands were not induced by Wnt-1 or Wnt-5a, but addition of metalloproteinase inhibitors blocked the stimulation of EGFR and ERK phosphorylation. Thus, Wnt activation of EGFR is apparently mediated by an increase in the availability of EGFR ligands, for example by cleavage from an inactive precursor molecule.

Ullrich and colleagues have implicated metalloproteinase cleavage of the erbB ligands heparin-binding epidermal growth factor (HB-EGF) and amphiregulin in EGFR signalling following G-protein-coupled receptor activation [16,17]. The Frizzled receptors through which Wnts act are 7-transmembrane domain receptors that are structurally related to other families of G-protein-coupled receptor, and consequently these observations have parallels with those of Civenni and colleagues. In the series of experiments by the latter authors, heparin-binding epidermal growth factor was expressed at higher levels than other erbB ligands, leading the authors to conclude that it is a likely candidate for mediating Wnt transactivation of the EGFR [3]. However, previous studies indicate that known erbB ligands commonly cause phosphorylation of multiple receptors [18], whereas Wnt-induced phosphorylation of erbB2 and erbB3 were not observed [3]. It will be interesting to investigate further the erbB ligand mediating

Wnt signalling and identify the particular metalloproteinase involved in its activation. Regardless of the specific ligand involved, the activation of only EGFR and not other erbB receptors has implications for the consequences of activation of this pathway by Wnt signalling, since the erbB receptors preferentially activate different combinations of signalling molecules and display different oncogenic potencies [15]. The EGFR homodimer transduces a relatively weak mitogenic stimulus and requires the additional presence of ligand for transformation [15]. In contrast, erbB2/erbB3 heterodimers have potent mitogenic and transforming activity [15].

Can the results obtained by Civenni and colleagues using mammary epithelial cells in culture be translated to normal mammary epithelium *in vivo*? The EGFR is essential for murine mammary development, and EGF and other ligands are potent inducers of ductal growth [19], but EGF does not stimulate lobuloalveolar differentiation [20]. In contrast, expression of Wnt-1 and other Wnt/ β -catenin pathway genes stimulates lobuloalveolar development and differentiation [4,11,12,21,22], producing a mammary structure that is morphologically distinct from that arising upon overexpression of EGFR [23]. Similarly, tumours arising from activation of the erbB and Wnt pathways in transgenic mice display distinct pathologies [24], although it should be noted that the erbB receptor used in these experiments was erbB2 rather than the EGFR. Further experimentation will be necessary to determine the relative balance between Wnt signalling via β -catenin and the EGFR in various model systems. Again, since various erbB ligand and receptor combinations have distinct roles in mammary development [19], the precise erbB ligands and receptors involved in Wnt signalling will also have implications for the role of Wnt-erbB crosstalk in mammary development and oncogenesis.

Downstream effectors: cyclin D1

Cyclin D1 is a central component of regulation of breast cancer cell proliferation and is frequently overexpressed in breast cancer [25]. It is a target for Wnt signalling and many other mitogenic signalling pathways, including those downstream of steroid hormones and receptor tyrosine kinases [25]. Civenni and colleagues show that Wnt expression in HC11 cells or treatment with conditioned medium from cells expressing Wnt-1 or -5A increases expression of cyclin D1 and that this can be reversed by inhibition of EGFR kinase activity or in the presence of metalloproteinase inhibitors [3]. Thus, although in breast cancers increased cyclin D1 expression is correlated with the presence of nuclear or cytoplasmic (potentially active) β -catenin [26], cyclin D1 induction via the EGFR predominates over its induction via β -catenin in these experiments.

Cyclin D1 is a weak mammary oncogene, as indicated by the long latency of tumours arising in MMTV-cyclin D1

transgenic mice [27]. While induction of cyclin D1 is thus a potential contributor to Wnt- and EGFR-mediated oncogenesis, the increased proliferation resulting from increased cyclin D1 expression is only one component of the oncogenic process [28]. Cyclin D1 is essential for mitogenic signalling through the EGFR in breast cancer cells [29]. However, although cyclin D1 is acknowledged as a critical target for Wnt signalling, it is not essential for Wnt-1-mediated mammary tumorigenesis [30], raising the possibility that Wnt activation of processes other than proliferation may be central to its oncogenic role. An alternative explanation is that other, related molecules may compensate for the absence of cyclin D1, thereby permitting proliferation. Consistent with that idea, cyclin D2 expression is increased in mammary tumours arising in MMTV-Wnt-1 mice [30], and in HC11 cells expressing Wnt-1 (but not Wnt-5A) [3]. The latter response is intriguing in the light of the previous observation that Wnt-5A failed to induce transformation under conditions where Wnt-1 was highly transforming [8]. The mechanisms for a Wnt-mediated increase in cyclin D2 in mammary epithelial cells are unknown, but in some cell types the bicoid-related transcription factor Pitx2 is rapidly induced by the Wnt/ β -catenin pathway and serves as a competence factor that is necessary for cyclin D2 gene induction [31]. The restricted pattern of Pitx2 expression suggests that this mechanism will be tissue-specific, but as the authors point out, it may serve as a prototype for mechanisms operating in other tissues.

Conclusion

The finding that Wnts activate erbB signalling in addition to stimulating the prototypic Wnt/ β -catenin signalling pathway is provocative, revealing a new level of regulation by members of the Wnt family and providing further impetus for increased interest in these and other developmental pathways in breast cancer. One question raised by these studies is the relation between the expression patterns of Wnts, the erbB ligands potentially mediating their effects, and the metalloproteinases necessary for Wnt-mediated activation of EGFR signalling in breast cancer, and whether any relations identified are correlated with outcome in breast cancer. These findings also emphasise the interconnected nature of many signalling pathways in breast cancer: crosstalk between Wnt and EGFR signalling is one example, but another example currently under intense investigation is the emerging link between steroid receptor and receptor tyrosine kinase signalling. In both cases, better understanding of these pathways is likely to have implications beyond the biology of breast cancer, by identifying potential new markers of disease outcome and therapeutic targets. As one example, the ability of sFRP-1, which is down-regulated in a proportion of breast cancers [9,32], to inhibit Wnt activation of EGFR signalling suggests that therapies aimed at increasing sFRP levels or activity may be effective.

Competing interests

None declared.

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References

1. Taipale J, Beachy PA: **The hedgehog and Wnt signalling pathways in cancer.** *Nature* 2001, **411**:349-354.
2. Polakis P: **Wnt signaling and cancer.** *Genes Dev* 2000, **14**:1837-1851.
3. Civenni G, Holbro T, Hynes NE: **Wnt1 and Wnt5a induce cyclin D1 expression through erbB1 transactivation in HC11 mammary epithelial cells.** *EMBO Rep* 2003, **4**:166-171.
4. Smalley M, Dale TC: **Wnt signaling and mammary tumorigenesis.** *J Mammary Gland Biol Neoplasia* 2001, **6**:37-52.
5. Briskin C, Heineman A, Chavarría T, Elenbaas B, Tan J, Dey SK, McMahon JA, McMahon AP, Weinberg RA: **Essential function of Wnt-4 in mammary gland development downstream of progesterone signaling.** *Genes Dev* 2000, **14**:650-654.
6. Nusse R, Varmus HE: **Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome.** *Cell* 1982, **31**:99-109.
7. Roelink H, Wagenaar E, Lopes da Silva S, Nusse R: **Wnt-3, a gene activated by proviral insertion in mouse mammary tumors, is homologous to int-1/Wnt-1 and is normally expressed in mouse embryos and adult.** *Proc Natl Acad Sci USA* 1990, **87**:4519-4923.
8. Wong GT, Gavin BJ, McMahon AP: **Differential transformation of mammary epithelial cells by Wnt genes.** *Mol Cell Biol* 1994, **14**:6278-6286.
9. Wong SC, Lo SF, Lee KC, Yam JW, Chan JK, Hsiao WL: **Expression of frizzled-related protein and Wnt-signalling molecules in invasive human breast tumours.** *J Pathol* 2002, **196**:145-153.
10. Li Y, Hively WP, Varmus HE: **Use of MMTV-Wnt-1 transgenic mice for studying the genetic basis of breast cancer.** *Oncogene* 2000, **19**:1002-1009.
11. Michaelson JS, Leder P: **β -catenin is a downstream effector of Wnt-mediated tumorigenesis in the mammary gland.** *Oncogene* 2001, **20**:5093-5099.
12. Imbert A, Eelkema R, Jordan S, Feiner H, Cowin P: **Δ N89 β -catenin induces precocious development, differentiation and neoplasia in mammary gland.** *J Cell Biol* 2001, **153**:555-568.
13. Schroeder JA, Troyer KL, Lee DC: **Cooperative induction of mammary tumorigenesis by TGF α and Wnts.** *Oncogene* 2000, **19**:3193-3199.
14. Schroeder JA, Adriance MC, McConnell EJ, Thompson MC, Pockaj B, Gendler SJ: **ErbB- β -catenin complexes are associated with human infiltrating ductal breast and murine mammary tumor virus (MMTV)-Wnt-1 and MMTV-c-Neu.** *J Biol Chem* 2002, **277**:22692-22698.
15. Yarden Y, Sliwkowski MX: **Untangling the ErbB signalling network.** *Nat Rev Mol Cell Biol* 2001, **2**:127-137.
16. Prenzel N, Zwick E, Daub H, Leserer M, Abraham R, Wallasch C, Ullrich A: **EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF.** *Nature* 1999, **402**:884-888.
17. Gschwind A, Hart S, Fischer OM, Ullrich A: **TACE cleavage of proamphiregulin regulates GPCR-induced proliferation and motility of cancer cells.** *EMBO J* 2003, **22**:2411-2421.
18. Beerli RR, Hynes NE: **Epidermal growth factor-related peptides activate distinct subsets of ErbB receptors and differ in their biological activities.** *J Biol Chem* 1996, **271**:6071-6076.
19. Stern DF: **ErbBs in mammary development.** *Exp Cell Res* 2003, **284**:89-98.
20. Coleman S, Silberstein GB, Daniel CW: **Ductal morphogenesis in the mouse mammary gland: evidence supporting a role for epidermal growth factor.** *Dev Biol* 1988, **127**:304-315.
21. Miyoshi K, Rosner A, Nozawa M, Byrd C, Morgan F, Landesman-Bollag E, Xu X, Seldin DC, Schmidt EV, Taketo MM, Robinson

- GW, Cardiff RD, Hennighausen L: **Activation of different Wnt/ β -catenin signaling components in mammary epithelium induces transdifferentiation and the formation of pilar tumors.** *Oncogene* 2002, **21**:5548-5556.
22. Edwards PA, Hiby SE, Papkoff J, Bradbury JM: **Hyperplasia of mouse mammary epithelium induced by expression of the Wnt-1 (int-1) oncogene in reconstituted mammary gland.** *Oncogene* 1992, **7**:2041-2051.
23. Edwards PA, Abram CL, Bradbury JM: **Genetic manipulation of mammary epithelium by transplantation.** *J Mammary Gland Biol Neoplasia* 1996, **1**:75-89.
24. Rosner A, Miyoshi K, Landesman-Bollag E, Xu X, Seldin DC, Moser AR, MacLeod CL, Shyamala G, Gillgrass AE, Cardiff RD: **Pathway pathology: histological differences between ErbB/Ras and Wnt pathway transgenic mammary tumors.** *Am J Pathol* 2002, **161**:1087-1097.
25. Musgrove EA, Hui R, Sweeney KJE, Watts CKW, Sutherland RL: **Cyclins and breast cancer.** *J Mammary Gland Biol Neoplasia* 1996, **1**:153-162.
26. Lin S-Y, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, Pestell RG, Hung M-C: **β -catenin, a novel prognostic marker for breast cancer: Its roles in cyclin D1 expression and cancer progression.** *Proc Natl Acad Sci USA* 2000, **97**:4262-4266.
27. Wang TC, Cardiff RD, Zukerberg L, Lees E, Arnold A, Schmidt EV: **Mammary hyperplasia and carcinoma in MMTV-cyclin D1 transgenic mice.** *Nature* 1994, **369**:669-671.
28. Hanahan D, Weinberg RA: **The hallmarks of cancer.** *Cell* 2000, **100**:57-70.
29. Lukas J, Bartkova J, Bartek J: **Convergence of mitogenic signalling cascades from diverse classes of receptors at the cyclin D-cyclin-dependent kinase-pRb-controlled G₁ checkpoint.** *Mol Cell Biol* 1996, **16**:6917-6925.
30. Yu Q, Geng Y, Sicinski P: **Specific protection against breast cancers by cyclin D1 ablation.** *Nature* 2001, **411**:1017-1021.
31. Kiuoussi C, Briata P, Baek SH, Rose DW, Hamblet NS, Herman T, Ohgi KA, Lin C, Gleiberman A, Wang J, Brault V, Ruiz-Lozano P, Nguyen HD, Kemler R, Glass CK, Wynshaw-Boris A, Rosenfeld MG: **Identification of a Wnt/Dvl/ β -Catenin \rightarrow Pitx2 pathway mediating cell-type-specific proliferation during development.** *Cell* 2002, **111**:673-685.
32. Ugolini F, Charafe-Jauffret E, Bardou VJ, Geneix J, Adelaide J, Labat-Moleur F, Penault-Llorca F, Longy M, Jacquemier J, Birnbaum D, Pebusque MJ: **WNT pathway and mammary carcinogenesis: loss of expression of candidate tumor suppressor gene SFRP1 in most invasive carcinomas except of the medullary type.** *Oncogene* 2001, **20**:5810-5817.

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