Commentary

The complexities of breast cancer desmoplasia

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Abstract

The stromal, or 'desmoplastic', responses seen histologically in primary breast carcinomas can vary from being predominantly cellular (fibroblasts/myofibroblasts) with little collagen to being a dense acellular tissue. The mechanisms underlying the stromal response are complex; paracrine activation of myofibroblasts by growth factors is important but the contribution of cytokines/chemokines should not be ignored. A recent xenograft study has proposed that platelet-derived growth factor (PDGF) is the initiator of the desmoplastic response, but this has not been confirmed by (limited) analyses *in vivo*. Further studies are required to elaborate the mechanisms of the desmoplastic response, to determine its role in breast cancer progression and whether it is the same for all carcinomas.

Keywords: breast cancer, desmoplasia, platelet-derived growth factor, stroma, transforming growth factor-β

Introduction

One of the features of many carcinomas, particularly breast, is the presence of a dense collagenous stroma, the so-called desmoplastic response, which can be responsible for the clinical presentation of a tumour as a 'lump'. Although studies in the 1950s proposed that the desmoplasia represented the condensation of pre-existing collagen [1] there is now good evidence that the collagen is synthesised by myofibroblasts present in the interstitium [2,3]. Several mechanisms that result in myofibroblast activation and collagen synthesis have been proposed. These include immune cytokine mechanisms and microvascular injury [4], with features analogous to wound healing [5], and paracrine activation of myofibroblasts by growth factors released by tumour cells [6,7]. Various growth factors, such as transforming growth factor (TGF)-α, TGF-β, insulin-like growth factor (IGF)-I, IGF-II and platelet-derived growth factor (PDGF), have been identified that are secreted by cancer cells and can stimulate stromal cells [8–11]. It is evident that complex epithelial–stromal cell interactions exist. Many of the data supporting this come from studies *in vitro*, which by their nature are generally short term.

Histological examination of a range of primary breast carcinomas shows that the stromal response can vary from being predominantly cellular (fibroblasts/myofibroblasts) with little collagenous tissue, through to a dense collagen stroma with apparently few stromal cells. The obvious question is why there are these differences and how the findings in primary breast carcinomas relate to cell-based co-culture systems *in vitro*. There is also a need to understand the nature of the role of the stromal desmoplasia in cancer progression: does it vary with the transition from disease *in situ* to invasive disease?

Xenograft model

To determine the mechanisms of development of desmoplasia a more appropriate model system is required. Previous studies of human tumour xenografts in 'nude' mice

IGF = insulin-like growth factor; PDGF = platelet-derived growth factor; TGF = transforming growth factor; TIMP = tissue inhibitor of metalloproteinases.

have shown a lack of desmoplastic response [12]. In a recent paper Shao *et al* [13] have described a model system aimed at reproducing the desmoplastic response in a xenograft system. Their hypothesis was that the established breast cancer lines used in such models had a rapid growth rate that might overwhelm any host stromal response, and that the cells had lost the expression of critical paracrine growth factors. To test this, they used a variety of oestrogen-receptor-positive and oestrogen-receptor-negative breast cancer cell lines plus three MCF-7 lines transfected with c-*ras*^{H} (W9 and W7) or the *neo* selectable marker. The *ras*^{H} transfection had previously been shown to reduce the growth dependence of cells for exogenous oestrogen, owing to increased secretion of different growth factors by the transfected cells [14]. The c-*ras* MCF-7 was also transfected with a PDGF-A dominant-negative mutant. Myofibroblasts cultured from desmoplastic breast carcinomas were treated with conditioned medium from all cell lines, and all lines were grown as xenografts in oophorectamised nude mice. All of the breast cancer xenografts grew as cellular tumours with little or no stroma formation apart from the tumour formed by W9 grown in the absence of oestrogen, which showed a marked desmoplastic response. Stromal cells comprised 30% of it, there was a high collagen content, and *in situ* hybridisation demonstrated stromelysin 3, tissue inhibitor of metalloproteinases (TIMP)-1 and IGF-II, with a zonal pattern of stromelysin 3 and TIMP-1, as described in primary breast carcinomas [15,16]. The authors suggest that the induced tumour parallels a grade I or II breast carcinoma, whereas the non-desmoplastic xenografts represent cancers of high histological grade. This extrapolation seems too simple because several of the cells used were oestrogen receptor positive, the presence of which in primary breast carcinomas relates to a better grade and stromal elastosis [17]. In this respect the model is limited in its reflection of human breast carcinomas. All of the cell lines examined expressed significant myofibroblast mitogenic activity, apart from MDA-MB-157. Analysis of conditioned medium from W9 cells revealed PDGF to be a major component of activity. In view of this, W9 was transfected with a PDGF-A dominant-negative mutant to determine whether PDGF was the factor causing the desmoplasia. Secretion of TGF-α, TGF-β_1, IGF-I and IGF-II was unaffected, but W9 cells with low PDGF, although tumorigenic, were non-desmoplastic in the absence of oestrogen. The conclusions drawn are that PDGF, secreted by the tumour cells and not host cells, and not other growth factors, is the primary initiator of the desmoplastic response, and that the mechanisms involved are paracrine rather than immune.

Primary breast carcinomas

This might be so in the model presented, which uses established human breast cancer cells injected into nude mice, but is it as simple as this in primary human breast carcinomas? Shao *et al* [13] acknowledge that other paracrine growth factors and inflammatory cell factors might contribute to tumour desmoplasia but propose that only PDGF is the major initiator. Examination of PDGF expression in (pre-invasive) ductal carcinoma *in situ* of different grades and in invasive carcinomas with differing stromal responses and behaviours would provide information about the role of PDGF. One study of PDGF-AA and PDGF-BB and their receptors in invasive carcinomas found expression of PDGF-AA and PDGF-BB in the cancer cells of 42% and 53% of cases, respectively, but only PDGFα receptor. Both growth factors and receptors were present in stromal cells of almost all cases and were co-expressed in proximity [18]. Although PDGF expression might have accounted for baseline stromal proliferation it did not readily explain differences in stromal proliferation between tumours. However, TGF-β_1 and TGF-β_2 and the TGF-β-receptor combination did show variable expression, which could indicate paracrine stromal stimulation, in one-third of cases. This could explain differences in stromal induction between tumours. There is other evidence for a role for TGF-β_1 in stromal deposition: a significant correlation has been found between the presence of TGF-β_1 in cancer cells and stromal fibronectin and tenascin [19].

The role of the desmoplastic reaction in breast cancer progression is still unclear, and it could vary depending on the nature of the reaction. At the stage of *in situ* carcinoma growth factors secreted by the malignant epithelial cells, either PDGF [13] or TGF-β_1 [20], or both, with or without other factors, could stimulate myofibroblasts within the adjacent stroma. These could synthesise a variety of stromal proteins (such as fibronectin, tenascin and collagens 1 and 3), metalloproteinases [21,22] and growth factors with angiogenic effects [23], which aid invasion, aid the subsequent growth of cancer cells and promote metastasis. An understanding of the nature of the growth factors involved in the stimulation of myofibroblasts is important for the development of inhibitors that could be used in the early stages of the disease. However, what is not clear is whether the dense collagenous stroma seen in a proportion of invasive breast carcinomas is promoted by the same stimuli, and whether it promotes or impedes breast cancer progression. If it is the latter, then identification of the mechanism of its formation, and whether there can be a transition from the tumour-enhancing cellular form to the dense impeding form, is important for the development of relevant therapeutic agents.

The evidence presented here favours paracrine growth factor mechanisms for the induction of the stromal response, but the role of chemokines [24] and connective tissue growth factor [25], for example, should not be ignored.

Conclusion

There is still much to understand about the desmoplastic response, with the need for a model that parallels the transition from *in situ* to invasive carcinoma and a more detailed evaluation of its significance in a range of primary breast carcinomas.

References

1. Jackson JG, Orr JW: **The ducts of carcinomatous breasts with particular reference to connective tissue changes.** *J Pathol Bacteriol* 1957, **74**:265–273.
2. Barsky SH, Rao CN, Grotendorst GR, Liotta LA: **Increased content of type V collagen in desmoplasia of human breast carcinoma.** *Am J Pathol* 1982, **108**:276–283.
3. Lagacé R, Grimaud J-A, Schürch, Seemayer TA: **Myofibroblastic stromal reaction in carcinoma of the breast: variations of collagenous matrix and structural glycoproteins.** *Virchows Arch* 1985, **408**:49–59.
4. Dvorak HF, Dickersin GR, Dvorak AM, Manseau EJ, Pyne K: **Human breast carcinoma: fibrin deposits and desmoplasia. Inflammatory cell type and distribution. Microvasculature and Infarction.** *J Natl Cancer Inst* 1981, **67**:335–345.
5. Dvorak HF: **Tumours: wounds that do not heal. Similarities between tumour stroma generation and healing.** *N Engl J Med* 1986, **315**:1650–1659.
6. Peres R, Betsholtz C, Westermark B, Heldin C-H: **Frequent expression of growth factors for mesenchymal cells in human mammary carcinoma cell lines.** *Cancer Res* 1987, **47**:3425–3429.
7. Ethier SP: **Growth factor synthesis and human breast cancer progression.** *J Natl Cancer Inst* 1995, **87**:964–973.
8. Horgan K, Jones DL, Mansel RE: **Mitogenicity of human fibroblasts in vivo for human breast cancer cells.** *Br J Surg* 1987, **74**: 227–229.
9. Ronnov-Jessin L, Petersen OW: **Induction of α-smooth muscle actin by transforming growth factor-β_1 in quiescent human breast gland fibroblasts.** *Lab Invest* 1993, **68**:696–707.
10. Ellis MJC, Singer C, Hornby A, Rasmussen A, Cullen KJ: **Insulin-like growth factor mediated stromal–epithelial interactions in human breast cancer.** *Breast Cancer Res Treat* 1994, **31**:249–261.
11. Bronzert DA, Pantazis P, Antoniades HN, Kasid A, Davidson N, Dickson RB, Lippman ME: **Synthesis and secretion of platelet derived growth factor by human breast cancer cell lines.** *Proc Natl Acad Sci USA* 1987, **84**:5763–5767.
12. Povlsen CO: **Heterotransplants of human tumours in nude mice.** *Antibiot Chemother* 1980, **28**:15–20.
13. Shao Z-M, Nguyen M, Barsky SH: **Human breast carcinoma desmoplasia is PDGF initiated.** *Oncogene* 2000, **19**:4337–4345.
14. Dickson RB, Kasid A, Huff KC, Bates SE, Knabbe C, Bronzert D, Gelmann EP, Lipmann ME: **Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17 β-estradiol or v^H-*ras* oncogene.** *Proc Natl Acad Sci USA* 1987, **84**: 837–841.
15. Basset P, Bellocq JP, Woy C, Stoll I, Huton P, Limacher JM, Podhajcer DL, Chenard MP, Rio MC, Chambon P: **A novel metalloproteinase gene specificity expressed in stromal cells of breast carcinomas.** *Nature* 1990, **348**:699–704.
16. Tomlinson J, Barsky SH, Nelson S, Singer S, Pezeshki B, Lee MC, Eilber F, Nguyen M: **Different patterns of angiogenesis in sarcomas and carcinomas.** *Clin Cancer Res* 1999, **5**:3516–3522.
17. Rasmussen BB, Rose C, Thorpe SM, Hou-Jenson K, Daehnfeldt JL, Palshof T: **Histopathological characteristics and oestrogen receptor content in primary breast carcinoma.** *Virchows Arch* 1981, **390**:347–351.
18. De Jong JS, van Diest PJ, van der Valk, Baak JPA: **Expression of growth factors, growth-inhibiting factors and their receptors in invasive breast cancer. I. An inventory in search of autocrine and paracrine loops.** *J Pathol* 1998,**184**:44–52.
19. Walker RA, Dearing SJ, Gallacher B: **Relationship of transforming growth factor β_1 to extracellular matrix and stromal infiltrates in invasive breast carcinomas.** *Br J Cancer* 1994, **69**: 1160–1165.
20. Walker RA, Dearing SJ: **Transforming growth factor β_1 expression in in-situ and invasive breast cancers.** *Eur J Cancer* 1992, **28**:641–644.
21. Ronnov-Jenson L, Petersen OW, Bissel M: **Cellular changes involved in the conversion of normal to malignant breast: importance of the stromal reaction.** *Physiol Rev* 1996, **76**: 69–125.
22. Jones JL, Glynn P, Walker RA: **Expression of MMP-2 and MMP-9, their inhibitors and the activator MT1-MMP in primary breast carcinomas.** *J Pathol* 1999, **189**:161–168.
23. De Jong JS, van Diest PJ, van der Valk P, Baak JPA: **Expression of growth factors, growth-inhibiting factors, and their receptors in invasive breast cancer. II. Correlations with proliferation and angiogenesis.** *J Pathol* 1998, **184**:53–57.
24. Devalaraja MN, Richmond A: **Multiple chemotactic factors: fine control or redundancy?** *Trends Pharmacol Sci* 1999, **20**:151–156.
25. Steffen CL, Ball-Mirth DK, Harding PA, Bhattacharyya N, Pillai S, Brigstock DR: **Characterization of cell associated and soluble forms of connective tissue growth factor produced by fibroblast cells in vitro.** *Growth Factors* 1998, **15**:199–123.