Commentary

The plasticity of human breast carcinoma cells is more than epithelial to mesenchymal conversion

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Abstract

The human breast comprises three lineages: the luminal epithelial lineage, the myoepithelial lineage, and the mesenchymal lineage. It has been widely accepted that human breast neoplasia pertains only to the luminal epithelial lineage. In recent years, however, evidence has accumulated that neoplastic breast epithelial cells may be substantially more plastic in their differentiation repertoire than previously anticipated. Thus, along with an increasing availability of markers for the myoepithelial lineage, at least a partial differentiation towards this lineage is being revealed frequently. It has also become clear that conversions towards the mesenchymal lineage actually occur, referred to as epithelial to mesenchymal transitions. Indeed, some of the so-called myofibroblasts surrounding the tumor may have an epithelial origin rather than a mesenchymal origin. Because myoepithelial cells, epithelial to mesenchymal transition-derived cells, genuine stromal cells and myofibroblasts share common markers, we now need to define a more ambitious set of markers to distinguish these cell types in the microenvironment of the tumors. This is necessary because the different microenvironments may confer different clinical outcomes. The aim of this commentary is to describe some of the inherent complexities in defining cellular phenotypes in the microenvironment of breast cancer and to expand wherever possible on the implications for tumor suppression and progression.

Keywords: breast cancer, differentiation programs, epithelial to mesenchymal transition, myoepithelial cells, myofibroblasts

Introduction

Human breast cancer cells are generally believed to originate from the luminal epithelial lineage of terminal duct lobular units [1]. Accordingly, the phenotype of most breast cancer cell lines is luminal, and they express sialomucin and keratin K19 [2,3] as well as other epithelial markers. In recent years, however, there has been an increasing appreciation of human breast carcinoma cells being more flexible in their differentiation program. Intra-tumor and inter-tumor heterogeneity of human breast cancer should no longer be viewed as a consequence of phenotypic drifting due to genetic instability, but also from the point of view of different differentiation repertoires available to the neoplastic cells in response to the tumor microenvironment, including reversion to a 'normal' phenotype (for an overview, see [4]). This is important because it allows for possible strategies to influence the breast cancer cells towards a more differentiated state (for a

EMT = epithelial to mesenchymal transition.

review, see [5]). We recently showed that the myoepithelial lineage is derived directly from the normal luminal epithelial lineage [6]. It has been observed previously that the luminal epithelial lineage of breast cancer was defective in its ability to differentiate along the myoepithelial differentiation program [7]. The growing number of myoepithelial markers is similarly providing evidence that neoplastic breast cancer cells frequently exhibit at least a partial myoepithelial differentiation program [8–10]. If the normal myoepithelial differentiation program is indeed tumor suppressive [5,11,12], then defining these traits becomes rather important for diagnosis and therapy. Furthermore, recent data based on loss of heterozygosity suggest that the neoplastic and mesenchymal compartments, of which the latter contain the myofibroblasts, are directly evolutionarily connected [13–15]. It has also been shown that loss of differentiation in breast cancer, invasion and metastasis both *in vivo* and in culture concur with epithelial to mesenchymal transition (EMT) of the tumor cells [16–21]. The fact that EMT-derived tumor cells and the myoepithelial lineage are both defined by expression of the same mesenchymal marker (i.e. vimentin), and furthermore that the myoepithelial lineage and peritumoral myofibroblasts share almost the same myodifferentiation program, warrants a more ambitious set of markers to define these cells and reveal the true implications of their presence *in vivo*.

Myoepithelial differentiation in breast neoplasia

Myoepithelial cells are ectodermally derived, as are luminal epithelial cells, and they rest between the basal side of the luminal cells and the basement membrane that they manufacture themselves [22]. They nevertheless express mesenchymal vimentin and Thy-1 along with certain 'basal' keratins including K5, K14 and K17 [1].

Over the years, the literature has accumulated the fact that the myoepithelial differentiation pathway is essentially absent in breast carcinomas. If the criteria used for identification of myoepithelial cells in breast cancer are the expression of a complete differentiation program, then it is correct that myoepithelial differentiation in breast cancer is an exception. Many studies have, however, focused exclusively on the expression of the basement membrane molecule laminin, which is usually absent in high-grade neoplastic breast cancer cells [23,24]. But if we look at some of the other myoepithelial markers, the picture changes radically. Both keratins K14 and K17 as well as vimentin have been reported present in 20–33% of invasive breast carcinomas and often at the epithelial–stromal junction, which is also the location of myoepithelial cells in the normal breast [25,26]. Remarkably, similar numbers have been reported for the frequency of ultrastructurally identified myoepithelial cells in breast cancer and for the expression of other markers such as oxytocin receptors, metallothionine, and connexin 43 [8–10,27]. This suggests that, even though breast cancer cells first and foremost express luminal epithelial keratins and sialomucin, some of the cells still retain their intrinsic ability to switch to a myoepithelial differentiation program [6], albeit expressing only partial changes towards a myoepithelial phenotype.

What is the potential consequence of myoepithelial differentiation in breast cancer? Basically, all myoepithelial-specific proteins tested in experimental tumor assays point in the same direction; namely, that they are tumor suppressive. The genes qualify as type II tumor suppressor genes [28], and include maspin [12], α6-integrin [29], cytokeratin 5 [30], connexin 43 [31], caveolin-1 [32], α-smooth muscle actin [33], and myoepithelium-derived serine proteinase inhibitor [34]. The soluble factors from normal myoepithelial cells such as activin [35] and relaxin [36] also inhibit growth of breast cancer cells and facilitate their differentiation. Furthermore, some factors (e.g. myoepithelial-specific CD44) were shown to be anti-angiogenic [37]. It has been experimentally difficult, however, to extrapolate from expression analyses in culture to the function of cancer-derived myoepithelial cells *in vivo*. To this end, precise assays for the functional phenotype of normal and abnormal breast myoepithelial cells are highly warranted.

Myoepithelial cells and myofibroblasts have many traits in common including vimentin and α-smooth muscle actin, and occasionally smooth muscle heavy-chain myosin [1]. It could thus be asked whether the plasticity of breast carcinoma could ever go further to result in the formation of true mesenchymal cells in an epithelial→myoepithelial→mesenchymal transition process. If this were the case, stromal myofibroblasts should occasionally express remnants of the myoepithelial phenotype, such as keratins. We have not so far been able to document such a differentiation program [38]. Others have, however, reported the occurrence of myofibroblasts with remnants of K14 expression (described as 'converted myoepithelial cells') in the central acellular zone of desmoplastic reactions [39]. Also, in a rat tumor progression series and in canine mixed mammary tumors, one of the steps in the evolution of mesenchymal cells involves the expression of typical myoepithelial traits [40]. Further studies are needed to elucidate how widespread this phenomenon is and to determine the possible consequences of such a conversion. It is clear that defining all these lineages based exclusively on mesenchymal markers such as vimentin may no longer be sufficient (see later).

Mesenchymal differentiation

Although vimentin is distinctly a part of the myoepithelial phenotype as already described, its presence in human carcinoma cells has mainly been attributed to a direct EMT

without myoepithelial intermediates (for example, see [19]). Originally, EMT was described in morphogenic remodelings during embryonic development (for reviews, see [41,42]). Later, it was shown to occur in cultured mammary epithelial cells [43] and in a bladder carcinoma cell line, NBT-II cells [44]. In culture, the definition is limited to the escape of single cells from epithelial sheets, increased motility, and a modification of the differentiation program so that the migrating cells no longer express epithelial characteristics, but acquire a mesenchymal phenotype [44]. More specifically, in NBT-II cells, the mesenchymal phenotype is – apart from the morphological change to spindle cells – defined by the acquisition of vimentin, while the loss of epithelial phenotype is defined by a decline in keratin and desmoglein expression; there is no change in the expression of E-cadherin or catenins. The epithelial phenotype is thus not completely lost, and a single marker defines the mesenchymal phenotype. This definition of EMT has been adopted in the field of breast cancer research.

Vimentin expression was originally found in hormone-independent cell lines [45]. This was later expanded to cell lines that were drug resistant [46]. This transition was monitored by a decline in keratin K19 and loss of E-cadherin, and a reduced expression of desmoplakins and occludins [46]. Similar criteria have been used in other laboratories as well as in one of ours, also with vimentin as the only molecular marker of a mesenchymal phenotype [16,47]. This definition of the mesenchymal phenotype is, however, similar to the myoepithelial phenotype. The definition of EMT in breast cancer, therefore, is much more demanding to address than in, for example, bladder cancer or colon cancer, where the myoepithelial program is not an option. Another possible definition of EMT in breast epithelial cells is the spread of cells in collagen gels [48]. We have previously shown that a subset of cells within the luminal epithelial lineage may convert to myoepithelial cells, and these have been shown to be very active in cell spread in collagen gels [6,49]. This definition is therefore also not exclusive. These observations underscore the general importance of further lineage characterization of spindle cells derived from cuboidal cells in the normal breast and in breast cancer. A simple keratin profiling of the mesenchymal-like cells should reveal whether they are the product of a direct EMT from the luminal lineage or whether they represent cells converted via the myoepithelial lineage. In the first case, remnants of the luminal phenotype should be present in the form of keratins K7, K8, K18 and possibly K19. In the latter case, basal keratins K5, K14 and K17 would be expected to dominate the profile. Such distinction is important because, as already stated, the biological and clinical implications of direct luminal epithelial EMT is very different from that deduced from the tumor suppressive myoepithelial phenotype.

Clinically, the concept of direct EMT is used to explain the phenotype of very aggressive metastatic cells to the bone marrow [50]. These cells express vimentin in conjunction with luminal epithelial markers [50]. Their aggressive phenotype concurs well with observations that vimentin-positive luminal breast carcinoma cells are invasive in cell culture assays [19]. For comparison, the vimentin-positive spindle cells of pure myoepitheliomas are considered essentially non-metastatic [51]. The complications in deciphering the mesenchymal phenotype become even more compelling when focus is turned from metastatic cells in the bone marrow to heterogeneous primary tumors that consist of many cell types. In primary tumors, there is no unequivocal correlation between gain of vimentin or loss of E-cadherin expression and poor prognosis [16,52]. One explanation for this could be that some of the cases with vimentin-positive cells and E-cadherin-negative cells reflect myoepithelial differentiation rather than direct EMT from luminal epithelial cells.

A stromal condition that has been overwhelmingly associated with poor prognosis in breast cancer is the desmoplastic reaction [53]. Desmoplasia is believed to be the result of excessive extracellular matrix formation as manufactured by peritumoral myofibroblasts [54]. As seen from the aforementioned considerations, however, the current definition of EMT does not incorporate the complete loss of keratins or gain of other mesenchymal markers other than vimentin (such as, for example, α-smooth muscle actin). Indeed, we have some evidence (Petersen et al., submitted for publication) that breast cancer cells, given the right conditions, may convert all the way to myofibroblasts. Also, it has been shown that typical and presumably myofibroblastic stroma of breast carcinomas frequently share the same loss of heterozygosity, with the epithelial neoplasia indicating a common cellular origin [13,14,55]. Stromal cells have also been shown to share p53 mutations with the carcinoma cells [14]. Moreover, a number of phenotypic traits such as IGFII, several transcription factors and protein kinases in myofibroblasts appear to be permanently overexpressed as if they were the result of typical genomic amplifications [56,57]. A consequence of a possible evolutionary relationship between the stroma and the neoplastic lesion remains to be seen.

Concluding remarks

Evidence for phenotypic plasticity of luminal epithelial cells to the other two lineages present in the normal breast is now compelling. Understanding breast carcinoma plasticity may be instrumental in attempts to take advantage of the tumor suppressiveness of myoepithelial differentiation or to interrupt EMT-dependent or desmoplasia-mediated progression. Thorough characterization of the epithelial and stromal compartments of normal breast and breast cancer need to be continued along with further emphasis

on development of culture model systems for epithelial–stromal interaction and conversions.

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References

1. Rønnov-Jessen L, Petersen OW, Bissell MJ: **Cellular changes involved in conversion of normal to malignant breast: The importance of the stromal reaction**. *Physiol Rev* 1996, **76**:69-125.
2. Bartek J, Taylor-Papadimitriou J, Miller N, Millis R: **Patterns of expression of keratin 19 as detected with monoclonal antibodies in human breast tissues and tumours**. *Int J Cancer* 1985, **36**:299-306.
3. Hilkens J, Buijs F, Hilgers J, Hageman P, Calafat J, Sonnenberg A, van der Valk M: **Monoclonal antibodies against human milk-fat globule membranes detecting differentiation antigens of the mammary gland and its tumors**. *Int J Cancer* 1984, **34**:197-206.
4. Bissell MJ, Weaver VM, Lelièvre SA, Wang F, Petersen OW, Schmeigel KL: **Tissue structure, nuclear organization, and gene expression in normal and malignant breast**. *Cancer Res* 1999, **59**:1757s-1764s.
5. Petersen OW, Rønnov-Jessen L, Weaver VM, Bissell MJ: **Differentiation and cancer in the mammary gland: Shedding new light on an old dichotomy**. *Adv Cancer Res* 1998, **75**:135-161.
6. Péchoux C, Gudjonsson T, Rønnov-Jessen L, Bissell MJ, Petersen OW: **Human mammary luminal epithelial cells contain progenitors to myoepithelial cells**. *Dev Biol* 1999, **206**:88-99.
7. Rudland PS, Hallowes RC, Cox SA, Ormerod EJ, Warburton MJ: **Loss of production of myoepithelial cells and basement membrane proteins but retention of response to certain growth factors and hormones by a new malignant human breast cancer cell strain**. *Cancer Res* 1985, **45**:3864-3877.
8. Ito Y, Kobayashi T, Kimura T, Matsuura N, Wakasugi E, Takeda T, Shimano T, Kubota Y, Nobunaga T, Makino Y, Azuma C, Kitamura Y, Saji F: **Investigation of the oxytocin receptor expression in human breast cancer tissue using newly established monoclonal antibodies**. *Endocrinology* 1996, **137**:773-779.
9. Jamieson S, Going JJ, D'Arcy R, George WD: **Expression of gap junction proteins connexin 26 and connexin 43 in normal human breast and in breast tumours**. *J Pathol* 1998, **184**:37-43.
10. Schmid KW, Ellis IO, Gee JMW, Darke BM, Lees WE, Kay J, Cryer A, Stark JM, Hittmair A, Öfner D, Dünser H, Margreiter R, Daxenbichler G, Nicholson RI, Bier B, Böcker W, Jasani B: **Presence and possible significance of immunocytochemically demonstrable metallothionein over-expression in primary invasive ductal carcinoma of the breast**. *Virch Arch A Pathol Anat* 1993, **422**:153-159.
11. Sternlicht MD, Kedeshian P, Shoa ZM, Safarians S, Barsky SH: **The human myoepithelial cell is a natural tumor suppressor**. *Clin Cancer Res* 1997, **3**:1949-1958.
12. Zou Z, Anisowicz A, Hendrix MJC, Thor A, Neveu M, Sheng S, Rafidi K, Seftor E, Sager R: **Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells**. *Science* 1994, **263**:526-529.
13. Wernert N, Hugel A, Locherbach C: **Genetic alterations in the fibroblastic stroma of invasive colon and breast carcinomas**. *Verh Dtsch Ges Pathol* 1998, **82**:317-321.
14. Wernert N, Locherbach C, Wellman A, Behrens P, Hugel A: **Presence of genetic alterations in microdissected stroma of human colon and breast cancers**. *J Mol Med* 2000, **78**:B30.
15. Moinfar F, Man Y-G, Bratthauer GL, Ratschek M, Tavassoli FA: **Genetic abnormalities in mammary ductal intraepithelial neoplasia-flat type ('clinging ductal carcinoma in situ')**. *Cancer* 2000, **88**:2072-2081.
16. Birchmeier C, Birchmeier W, Brand-Saberi B: **Epithelial–mesenchymal transitions in cancer progression**. *Acta Anat* 1996, **156**:217-226.
17. Boyer B, Vallés AM, Edme N: **Induction and regulation of epithelial–mesenchymal transitions**. *Biochemical Pharmacology* 2000, **60**:1091-1099.
18. Thiery JP, Chopin D: **Epithelial cell plasticity in development and tumor progression**. *Cancer Metastasis Rev* 1999, **18**:31-42.
19. Gilles C, Polette M, Birembaut P, Brunner N, Thompson EW: **Expression of c-ets-1 mRNA is associated with an invasive, EMT-derived phenotype in breast carcinoma cell lines**. *Clin Exp Metastasis* 1997, **15**:519-526.
20. Gilles C, Thompson EW: **The epithelial to mesenchymal transition and metastatic progression in carcinoma**. *Breast J* 1996, **2**:83-96.
21. Oft M, Heider K-H, Beug H: **TGFβ signaling is necessary for carcinoma cell invasiveness and metastasis**. *Curr Biol* 1998, **8**:1243-1252.
22. Hamperl H: **The myothelia (myoepithelial cells). Normal state; regressive changes; hyperplasia; tumors**. In *C. T. in Pathology*. Heidelberg: Springer-Verlag, 1970:161-220.
23. Albrechtsen R, Nielsen M, Wewer U, Engvall E, Ruoslahti E: **Basement membrane changes in breast cancer detected by immunohistochemical staining for laminin**. *Cancer Res* 1981, **41**:5076-5081.
24. Gusterson BA, Warburton MJ, Mitchell D, Ellison M, Neville AM, Rudland PS: **Distribution of myoepithelial cells and basement membrane proteins in the normal breast and in benign and malignant breast diseases**. *Cancer Res* 1982, **42**:4763-4770.
25. Wetzels RHW, Holland R, van Haelst UJGM, Lane B, Leigh IM, Ramaekers FCS: **Detection of basement membrane components and basal cell keratin 14 in noninvasive and invasive carcinomas of the breast**. *Am J Pathol* 1989, **134**:571-579.
26. Wada T, Yasutomi M, Hashmura K, Kunikata M, Tanaka T, Mori M: **Vimentin expression in benign and malignant lesions in the human mammary gland**. *Anticancer Res* 1992, **12**:1973-1982.
27. Hayashi Y, Aoki Y, Eto R, Tokuoka S: **Findings of myoepithelial cells in human breast cancer. Ultrastructural and immunohistochemical study by means of anti-myosin antibody**. *Acta Pathol Jpn* 1984, **34**:537-552.
28. Sager R: **Expression genetics in cancer; Shifting the focus from DNA to RNA**. *Proc Natl Acad Sci USA* 1997, **94**:952-955.
29. Sager R, Anisowicz A, Neveu M, Liang P, Sotiropoulou G: **Identification by differential display of alpha 6 integrin as a candidate tumor suppressor gene**. *FASEB J* 1993, **7**:964-970.
30. Zajchowski DA, Band V, Trask DK, Kling D, Connolly JL, Sager R: **Suppression of tumor-forming ability and related traits in MCF-7 human breast cancer cells by fusion with immortal mammary epithelial cells**. *Proc Natl Acad Sci USA* 1990, **87**:2314-2318.
31. Hirschi KK, Xu C, Tsukamoto T, Sager R: **Gap junction genes Cx26 and Cx43 individually suppress the cancer phenotype of human mammary carcinoma cells and restore differentiation potential**. *Cell Growth Diff* 1996, **7**:861-870.
32. Lee SW, Reimer CL, Oh P, Campbell DB, Schnitzer JE: **Tumor cell growth inhibition by caveolin re-expression in human breast cancer cells**. *Oncogene* 1998, **16**:1391-1397.
33. Okamoto-Inoue M, Kamada S, Kimura G, Taniguchi S: **The induction of smooth muscle α actin in a transformed rat cell line suppresses malignant properties in vitro and in vivo**. *Cancer Lett* 1999, **142**:173-178.
34. Xiao G, Liu YE, Gentz R, Sang QA, Ni J, Goldberg ID, Shi Y E: **Suppression of breast cancer growth and metastasis by a serpin myoepithelium-derived serine proteinase inhibitor expressed in the mammary myoepithelial cells**. *Proc Natl Acad Sci USA* 1999, **96**:3700-3705.
35. Liu QY, Niranjan B, Gomes P, Gomm JJ, Davies D, Coombes RC, Buluwela L: **Inhibitory effects of activin on the growth and morphogenesis of primary and transformed mammary epithelial cells**. *Cancer Res* 1996, **56**:1155-1163.
36. Bani D, Riva A, Bigazzi M, Sacchi BT: **Differentiation of breast cancer cells in vitro is promoted by the concurrent influence of myoepithelial cells and relaxin**. *Br J Cancer* 1994, **70**:900-904.
37. Alpaugh ML, Lee MC, Nguyen M, Deato M, Dishakjian L, Barsky SH: **Myoepithelial-specific CD44 shedding contributes to the anti-invasive and antiangiogenic phenotype of myoepithelial cells**. *Exp Cell Res* 2000, **261**:150-158.

38. Rønnov-Jessen L, Petersen OW, Koteliansky VE, Bissell MJ: **The origin of the myofibroblasts in breast cancer: Recapitulation of tumor environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells**. *J Clin Invest* 1995, **95**:859-873.
39. Tsuda H, Takarabe T, Hasegawa F, Fukutomi T, Hirohashi S: **Large, central acellular zones indicating myoepithelial tumor differentiation in high-grade invasive ductal carcinomas as markers of prediposition to lung and brain metastasis**. *Am J Surg Pathol* 2000, **24**:197-202.
40. Gartner F, Geraldes M, Cassali G, Rema A, Schmitt F: **DNA measurement and immunohistochemical characterization of epithelial and mesenchymal cells in canine mixed mammary tumors: putative evidence for a common histogenesis**. *Vet J* 1999, **158**:39-47.
41. Hay ED: **An overview of epithelio-mesenchymal transformation**. *Acta Anat* 1995, **154**:8-20.
42. Boyer B, Valles AM, Thiery JP: **Model systems of epithelium–mesenchyme transitions**. *Acta Anat* 1996, **156**:227-239.
43. Stoker M, Gherardi E, Perryman M, Gray J: **Scatter factor is a fibroblast-derived modulator of epithelial cell mobility**. *Nature* 1987, **327**:239-242.
44. Boyer B, Tucker GC, Vallés AM, Franke WW, Thiery JP: **Rearrangement of desmosomal and cytoskeletal proteins during the transition from epithelial to fibroblastoid organization in cultured rat bladder carcinoma cells**. *J Cell Biol* 1989, **109**:1495-1509.
45. Sommers CL, Walker-Jones D, Heckford SE, Worland P, Valverius E, Clark R, McCormick F, Stampfer M, Abularach S, Gelmann EP: **Vimentin rather than keratin expression in some hormone-independent breast cancer cell lines and in oncogene-transformed mammary epithelial cells**. *Cancer Res* 1989, **49**:4258-4263.
46. Sommers CL, Heckford SE, Skerker JM, Worland P, Torri JA, Thompson EW, Byers SW, Gelmann EP: **Loss of epithelial markers and acquisition of vimentin expression in adriamycin- and vinblastine-resistant human breast cancer cell lines**. *Cancer Res* 1992, **52**:5190-5197.
47. Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ: **Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that lead to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells**. *J Cell Biol* 1997, **29**:1861-1872.
48. Baeckstrom D, Lu PJ, Taylor-Papadimitriou J: **Activation of alpha2beta1 integrin prevents c-erbB2-induced scattering and apoptosis of human mammary epithelial cells in collagen gel**. *Oncogene* 2000, **19**:4592-4603.
49. Rudland PS, Ollerhead GE, Platt-Higgins AM: **Morphogenetic behaviour of simian virus 40-transformed human mmamry epithleial stem cell lines on collagen gels**. *In Vitro Cell Dev Biol* 1991, **27A**:103-112.
50. Putz E, Witter PE, Offner S, Stosiek P, Zippelius A, Johnson J, Riethmuller ZA, Pantel K: **Phenotypic characteristics of cell lines derived from dessiminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases**. *Cancer Res* 1999, **59**:241-248.
51. Foschini MP, Eusebi V: **Carcinomas of the breast showing myoepithelial cell differentiation**. *Virchows Arch* 1998, **432**:303-310.
52. Sesradi R, Raymond WA, Leong AS, Horsfall DJ, McCaul K: **Vimentin expression is not associated with poor prognosis in breast cancer**. *Int J Cancer* 1996, **67**:353-356.
53. Hasebe T, Tsuda H, Hirohashi S, Shimosato Y, Tsubono Y, Yamamoto H, Mukai K: **Fibrotic focus in infiltrating ductal carcinoma of the breast: a significant histopathological prognostic parameter for predicting the long-term survival of the patients**. *Breast Cancer Res Treat* 1998, **49**:195-208.
54. Shao Z-M, Nguyen M, Barsky SH: **Human breast carcinoma desmoplasia is PDGF initiated**. *Oncogene* 2000, **19**:4337-4345.
55. Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratchek M, Tavassoli F: **Concurrent and independent genetic alterations in the stromal and epithelial cells of the mammary carcinoma: Implications for tumorigenesis**. *Cancer Res* 2000, **60**:2562-2566.
56. Spanakis E, Brouty-Boye D: **Quantitative variation of proto-oncogene and cytokine gene expression in isolated breast fibroblasts**. *Int J Cancer* 1995, **29**:698-705.
57. Singer C, Rasmussen A, Smith HS, Lippman ME, Lynch HT, Cullen KJ: **Malignant breast epithelium selects for insulin-like growth factor II expression in breast stroma: evidence for paracrine function**. *Cancer Res* 1995, **55**:2448-2454.