## *Review*

# The role of cancer-associated fibroblasts in breast cancer pathobiology

**Yoon Yang Jung, Hye Min Kim and Ja Seung Koo**
Department of Pathology, Yonsei University College of Medicine, Seoul, South Korea

**Summary.** The role of the tumor microenvironment (TME) is critical in cancer pathobiology. Of the components of the TME, cancer-associated fibroblasts (CAFs) play a major role. Breast cancer is a typical tumor type, forming abundant tumor stroma, and CAFs are involved in various aspects of breast cancer, including carcinogenesis, tumor progression, invasion, metastasis, inflammation, metabolism, therapy resistance, and prognosis. Various factors, such as growth factors, cytokines, hormones secreted from CAFs, paracrine effects promoted by the extracellular matrix (ECM), and mechanical pressure, are involved in cancer development, and there are various crosstalk and signaling pathways among CAFs, cancer cells, epithelial cells, and the ECM. Recent studies have evaluated the potential of CAFs as therapeutic targets in breast cancer. In this review, we discuss the role of CAFs and their clinical implications.

**Key words:** Breast cancer, Cancer-associated fibroblast, Tumor microenvironment

## Introduction

With the progression of cancer research studies, the importance of the tumor microenvironment (TME) is becoming clear. The TME is a nontumor, non-transformed element present in the region surrounding the tumor and includes immune system elements (such as macrophages and lymphocytes), blood vessel cells, fibroblasts, myofibroblasts, mesenchymal stem cells, adipocytes, and extracellular matrix (ECM). In breast cancers, the TME plays an important role in tumor formation, the progression from ductal carcinoma in situ (DCIS) to invasive carcinoma, and metastasis (Hu et al., 2008; Mao et al., 2013). The tumor:stroma ratio and stroma type are associated with the recurrence, distance metastasis, and survival of breast cancer, indicating the importance of the TME in breast cancer (de Kruijf et al., 2011; Qian et al., 2011a,b).

Of the elements that form the TME, the most important and most extensively investigated are cancer-associated fibroblasts (CAFs) (Hu et al., 2008). CAFs are located adjacent to cancer cells and are involved in tumor initiation, tumor-stimulatory inflammation, metabolism, metastasis, drug response, and immune surveillance (Mao et al., 2013). In breast cancers, CAFs are the most common component of the tumor stroma and are involved in the determining tumor biology. Recently, preclinical and clinical trials have been performed to determine the effects of targeting CAFs in various tumors (Gonda et al., 2010).

In this review, we will discuss the various roles of CAFs in breast cancer and the clinical implications of CAFs in the development of anticancer therapies.

## Origin and phenotypes of CAFs

Although CAFs play an important role in tumor biology by crosstalk with tumor cells, there is no clear definition of CAFs because the cellular origin and markers of CAFs are unclear. CAFs have been proposed to originate from resident fibroblasts (Moskovits et al., 2006; Kojima et al., 2010), bone marrow-derived mesenchymal stem cells, cancer cells undergoing the epithelial-mesenchymal transition (EMT) (Petersen et al., 2003), endothelial cells undergoing the endothelial-mesenchymal transition (Zeisberg et al., 2007), and adipocytes (Jotzu et al., 2010).

Like tumor cells, the TME is heterogeneous owing to the existence of multiple subpopulations of fibroblasts. Therefore, there have been efforts to identify specific markers of CAFs with different characteristics, including the positive markers α-smooth muscle actin (SMA) (Huang et al., 2010), CD10 (Desmedt et al., 2012), podoplanin (Schoppmann et al., 2012), fibroblast activation protein (FAP) (Park et al., 1999), tenascin-C (Spenle et al., 2015), platelet-derived growth factor receptor (PDGFR) α/β (Forsberg et al., 1993; Shao et al., 2000), and fibroblast-specific protein 1 (FSP1) (O'Connell et al., 2011) and the negative markers caveolin-1 (Mercier et al., 2008) and laminin (Tlsty, 2001). Each specific CAF phenotype has unique characteristics depending on variable marker expression, with $CAF^{FAP}$, $CAF^{FSP1}$, $CAF^{PDGFR\alpha}$, and $CAF^{PDGFR\beta}$ representing the most common phenotypes (Cortez et al., 2014). The $CAF^{FAP}$ phenotype is associated with characteristics of activated CAFs, including invasive and immunomodulatory functions, whereas the $CAF^{FSP1}$ phenotype is associated with metastatic colonization and macrophage infiltration. Moreover, the $CAF^{PDGFR\alpha}$ phenotype is associated with angiogenesis and macrophage recruitment, and the $CAF^{PDGFR\beta}$ phenotype is associated with metastatic spread and high interstitial fluid pressure. Therefore, it is difficult to clearly define CAFs because of variability in their cellular origins and markers. Some authors have defined CAFs as a dynamic status of fibroblast-like cells in the tumor region, called 'CAF status' (Madar et al., 2013).

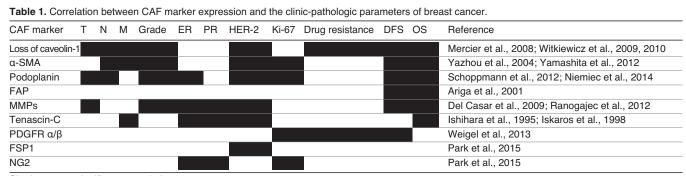## Signaling pathways mediating the interaction between breast cancer cells and CAFs

CAFs must be activated to affect breast cancer cells; this process involves multiple molecular signaling pathways. First, the autocrine signaling loop of tumor cell-derived factors, such as transforming growth factor (TGF)-β and CXCL12/SDF-1, activate CAFs (Kojima et al., 2010). Next, molecules secreted from cancer cells, such as PDGF-α/β (Shao et al., 2000), basic fibroblast growth factor (bFGF) (Strutz et al., 2000), and interleukin (IL)-6 (Hugo et al., 2012), can activate resident fibroblasts. Finally, downregulation of tumor-suppressor genes, such as p53 (Moskovits et al., 2006), p21 (Trimis et al., 2008), PTEN (Trimboli et al., 2009), caveolin-1 (Trimmer et al., 2011), and $p16^{INK4A}$ (Al-Ansari et al., 2013), by CAFs can induce procarcinogenic effects in breast stromal fibroblasts.

## Effects of CAFs on breast cancer

CAFs influence breast cancer cells through various mechanisms and are involved in the initiation, progression, metastasis, therapeutic effects, and prognosis of breast cancer. The associations between CAF markers and breast cancer clinicopathological characteristics are summarized in Table 1.

### Carcinogenesis

CAFs can be activated by interactions with cancer cells. However, several reports have proposed that CAFs can also mediate carcinogenesis, even before the existence of cancer cells. In a previous study, several proteins extracted from normal fibroblasts were shown to be involved in breast carcinogenesis (Fleming et al., 2010). Additionally, normal fibroblasts from reduction mammoplasty have been shown to have tumorigenic potency in vitro and in vivo (Dumont et al., 2013). In animal models, genetic manipulation or irradiation of fibroblasts increases cancer incidence (Trimboli et al., 2009; Cichon et al., 2010; Nguyen et al., 2011). In addition, inoculation of breast cancer cells (MCF-7 and

**Table 1.** Correlation between CAF marker expression and the clinic-pathologic parameters of breast cancer.

| CAF marker | T | N | M | Grade | ER | PR | HER-2 | Ki-67 | Drug resistance | DFS | OS | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Loss of caveolin-1 | ■ | ■ | ■ | ■ |  |  | ■ | ■ |  | ■ | ■ | Mercier et al., 2008; Witkiewicz et al., 2009, 2010 |
| α-SMA |  | ■ | ■ | ■ |  |  | ■ | ■ |  |  | ■ | Yazhou et al., 2004; Yamashita et al., 2012 |
| Podoplanin | ■ | ■ |  | ■ | ■ |  | ■ |  |  |  | ■ | Schoppmann et al., 2012; Niemiec et al., 2014 |
| FAP |  |  |  |  |  |  |  |  |  |  | ■ | Ariga et al., 2001 |
| MMPs | ■ |  |  | ■ | ■ | ■ | ■ |  |  |  | ■ | Del Casar et al., 2009; Ranogajec et al., 2012 |
| Tenascin-C |  | ■ |  | ■ | ■ |  |  |  |  | ■ |  | Ishihara et al., 1995; Iskaros et al., 1998 |
| PDGFR α/β |  |  |  |  |  |  |  | ■ | ■ | ■ |  | Weigel et al., 2013 |
| FSP1 |  |  |  |  |  |  | ■ |  |  |  |  | Park et al., 2015 |
| NG2 |  |  |  | ■ | ■ |  | ■ | ■ |  |  |  | Park et al., 2015 |

Shadow mean significant association.

MDA-MB-231) with CAFs has been shown to promote breast cancer development compared with that of breast cancer cell inoculation alone (Trimis et al., 2008). The mechanisms through which CAFs influence breast carcinogenesis include secretion of mutagenic materials, such as reactive oxygen species, and inactivation of tumor-suppressor genes. Indeed, a previous study showed that hyperactivation of PTEN in CAFs suppressed the role of CAFs in tumor initiation (Trimboli et al., 2009).

### Breast cancer progression

CAFs contribute to breast cancer progression through paracrine effects by secreting various substances. First, CAFs secrete various growth factors, such as hepatic growth factor (HGF), epidermal growth factor (EGF), bFGF, and insulin-like growth factor (IGF), which are involved in breast cancer progression (Tyan et al., 2011; Locatelli et al., 2012). Second, CAFs secretes cytokine. For example, SDF-1 activates CAFs and acts on tumor cell progression by cross-reacting with CXCR4 on the cancer cell surface (Orimo et al., 2005; Kojima et al., 2010). Additionally, CAFs secrete hormones and are a source of local estrogen (Sasano et al., 2010). Moreover, CAFs release cancer-associated aromatase and promote tumor cell progression (Yamaguchi et al., 2005; Miki et al., 2007).

### Breast cancer cell invasion and metastasis

CAFs play a key role in invasion and metastasis in breast cancers. Studies have shown that CAFs promote the EMT (Soon et al., 2013), which involves molecules such as TWIST (Lee et al., 2015) and SNAIL (Desmedt et al., 2012). CAFs also promote ECM degradation by matrix metalloproteinases (MMPs) and plasminogen activator (Kessenbrock et al., 2010), leading to modulation of cancer cell motility and the EMT through the degradation of growth factors and cytokines (Przybylo et al., 2007; Roy et al., 2009). Secretion of vascular endothelial growth factor by CAFs promotes angiogenesis and invasion (Hu et al., 2009). Additionally, CAFs are associated with the formation of a mechanical gradient pressure or force during tumorigenesis when CAFs are recruited and migrate from the tumor margin into the tumor center region; this force may promote tumor invasion (Karagiannis et al., 2012). Lastly, CAFs promotes the survival of circulating tumor cells (CTCs) by inducing circulating tumor cell stemness (Mani et al., 2008). Moreover, CAFs are involved in the invasion and metastasis of breast cancer, as well as organ-specific metastasis. For example, CAFs have been reported to be present in 80% of cases of brain metastasis associated with primary breast cancer (Duda et al., 2010) and to secrete CCL5, which stimulates $CD4^+$ $FOXP3^+$ regulatory T cells to promote lung metastasis of primary breast cancer (Tan et al., 2011).

### Drug resistance

Breast cancer is treated with various chemotherapies, including endocrine therapy and molecular targeted therapy, and CAFs are involved in mediating drug resistance to these therapies. Collagen type I, which is secreted by CAFs, is involved in chemotherapy resistance (Loeffler et al., 2006); specifically, chemotherapy-induced DNA damage in fibroblast induces the secretion of WNT16B, activates nuclear factor (NF)-ϰB, and induces mitoxantrone resistance (Johnson et al., 2013). Moreover, induction of HMGB1 expression by CAF is involved in doxorubicin resistance (Amornsupak et al., 2014). Several mechanisms involved in resistance to tamoxifen, a type of endocrine therapy, have been described, including mitogen-activated protein kinase (MAPK) and Akt hyperactivation by CAFs (Shekhar et al., 2007), CAF-

**Table 2.** CAF targeted anti-cancer therapy.

| Action mechanism | Target | Drug | Types of cancer | References |
|---|---|---|---|---|
| CAF differentiation | DNMT1 | 5-Aza-2′-deoxycytidine | Leukemia, lung cancer | Thottassery et al., 2014 |
| | FAPα | Sibrotuzumab | Colon cancer, lung cancer | Scott et al., 2003 |
| CAF-cancer cell interaction | HGF/Met | NK4 gene therapy | Mesothelioma, gastric cancer | Zhu et al., 2014; Tada et al., 2015 |
| | | Anti-HGF mAbs | Glioma | Kim et al., 2006 |
| | MMPs | Non-peptidic MMP inhibitors, TIMPs | Advanced cancer | Molina et al., 2005 |
| | SDF1/CXCL12 | AMD-3100 | Skin cancer | Sarchio et al., 2014 |
| | Smo | IPI-926 | Sarcoma | Campbell et al., 2014 |
| CAF-ECM interaction | PAI-1/uPAR | Urokinase-derived peptide (A6) | Ovarian cancer | Gold et al., 2012 |
| CAF-endothelial interaction | PDGF-C | Antibodies used with anti-VEGF-A | Renal cell carcinoma | Crawford et al., 2009 |
| CAF targeted anti-inflammatory signaling | CD11b+myeloid stromal cells | CTL | Brain tumor | Zhang et al., 2008 |
| | COX-2 | Celecoxib, Rofecoxib | Colon cancer | Buecher et al., 2005 |

mediated mitochondrial dysfunction (Martinez-Outschoorn et al., 2011), EGF receptor (EGFR) and phosphoinositol 3-kinase (PI3K)/AKT pathway activation (Pontiggia et al., 2012), and the EMT, promoted by secretion of inflammatory cytokines from CAFs (Mao et al., 2013). CAFs are also involved in target resistance. In triple-negative breast cancer (TNBC), HGF secreted by CAFs activates MET and induces resistance to gefitinib, an EGFR inhibitor (Mueller et al., 2012).

*Immune and metabolic alterations*

CAFs induce tumor-associated inflammation. Because CAFs release various cytokines and chemokines, they attract immune cells to the TME (Galdiero et al., 2013; Raz et al., 2013). Among mediators secreted by CAFs, CCL2 (Qian, et al., 2011), IL-6, tumor necrosis factor (TNF), and SDF-1 (Raz et al., 2013) are involved in immune cell recruitment. These mediators also affect the functional differentiation of immune cells.

CAFs are also involved in cancer metabolism in breast cancer. In general, the metabolism of malignant neoplasms can be explained by the Warburg effect theory, which is a metabolic shift towards glycolysis rather than oxidative phosphorylation (OXPHOS) in the mitochondria (Warburg, 1956). However, in breast cancers, there is metabolic crosstalk between the tumor cells and stromal cells, called the reverse Warburg effect theory (Pavlides et al., 2009; Bonuccelli et al., 2010; Martinez-Outschoorn et al., 2010a; Pavlides et al., 2010). In breast cancer cells, reactive oxygen species (ROS), such as nitric oxide (NO), are generated, and stromal cells are subjected to oxidative stress, resulting in glycolysis, autophagy (mitophagy), and mitochondrial dysfunction through hypoxia-inducible factor (HIF)-1$\alpha$ and NF-$\varkappa$B pathways. Pyruvate and lactate generated by glycolysis in stromal cells enters into tumor cells and produces ATP through OXPHOS, thereby promoting the survival and growth of tumor cells. In breast cancer, CAFs exhibiting loss of caveolin-1 expression owing to degradation by autophagy, are thought to interact with tumor cells (Pavlides et al., 2009, Martinez-Outschoorn et al., 2010, 2011).

*Breast cancer prognosis*

CAFs are involved in the initiation, progression, metastasis, and therapeutic effects of breast cancer and are therefore expected to be associated with prognosis. Studies have shown that the expression of CAF markers is associated with poor prognosis and tumor aggressiveness. In breast cancers, the expression of ECM-related genes (Bergamaschi et al., 2008) and tumor stroma histologic type are associated with prognosis (Ahn et al., 2012), suggesting that the tumor stroma is an important factor affecting breast cancer prognosis.

## Future targets for breast cancer treatment

Because CAFs affect a wide range of processes, including initiation, progression, invasion, metastasis, and therapy resistance, and are more genetically stable than tumor cells, therapies that target CAFs may be an effective approaches for treating breast cancer (Tchou and Conejo-Garcia, 2012). Therapeutic agents can target either the CAFs directly or the interaction between CAFs and other cells/components, such as cancer cells, the ECM, and endothelial cells. Many of these types of agents are under preclinical or clinical evaluation (Gonda et al., 2010).

## Conclusion

CAFs are a main component of the TME and originate from various types of cells. Moreover, CAFs express a range of different markers; therefore, the definition of CAFs has not yet been clearly established. Because breast cancers have an abundant tumor stroma, CAFs, the main component of the tumor stroma, affect the tumor biology of breast cancer and are involved in the carcinogenesis, progression, invasion, metastasis, inflammation, metabolism, therapy resistance, and prognosis of breast cancer. Various factors, including growth factors, cytokines, hormones released by CAFs, paracrine effects of the ECM, and mechanical pressure, are involved in the mediation of breast cancer biology by CAFs. Multiple crosstalk and signaling pathways mediate the interactions among CAFs, cancer cells, epithelial cells, and the ECM. Therefore, targeting of CAFs may represent a novel anticancer strategy, and various agents are in preclinical and clinical trials. However, the use of these agents may be limited because of the diversity of biological pathways active in CAFs, with lack of a specific, predominant pathway identified.

## References

Ahn S., Cho J., Sung J., Lee J.E., Nam S.J., Kim K.M. and Cho E.Y. (2012). The prognostic significance of tumor-associated stroma in invasive breast carcinoma. Tumour Biol. 33, 1573-1580.

Al-Ansari M.M., Hendrayani S.F., Shehata A.I. and Aboussekhra A. (2013). p16(INK4A) represses the paracrine tumor-promoting effects of breast stromal fibroblasts. Oncogene 32, 2356-2364.

Amornsupak K., Insawang T., Thuwajit P., O-Charoenrat P., Eccles S.A. and Thuwajit C. (2014). Cancer-associated fibroblasts induce high mobility group box 1 and contribute to resistance to doxorubicin in

breast cancer cells. BMC Cancer 14, 955.

Ariga N., Sato E., Ohuchi N., Nagura H. and Ohtani H. (2001). Stromal expression of fibroblast activation protein/seprase, a cell membrane serine proteinase and gelatinase, is associated with longer survival in patients with invasive ductal carcinoma of breast. Int. J. Cancer 95, 67-72.

Bergamaschi A., Tagliabue E., Sorlie T., Naume B., Triulzi T., Orlandi R., Russnes H.G., Nesland J.M., Tammi R., Auvinen P., Kosma V.M., Menard S. and Borresen-Dale A.L. (2008). Extracellular matrix signature identifies breast cancer subgroups with different clinical outcome. J. Pathol. 214, 357-367.

Bonuccelli G., Tsirigos A., Whitaker-Menezes D., Pavlides S., Pestell R.G., Chiavarina B., Frank P.G., Flomenberg N., Howell A., Martinez-Outschoorn U.E., Sotgia F. and Lisanti M.P. (2010). Ketones and lactate "fuel" tumor growth and metastasis: Evidence that epithelial cancer cells use oxidative mitochondrial metabolism. Cell Cycle 9, 3506-3514.

Buecher B., Bouancheau D., Broquet A., Bezieau S., Denis M.G., Bonnet C., Heymann M.F., Jarry A., Galmiche J.P. and Blottiere H.M. (2005). Growth inhibitory effect of celecoxib and rofecoxib on human colorectal carcinoma cell lines. Anticancer Res. 25, 225-233.

Campbell V.T., Nadesan P., Ali S.A., Wang C.Y., Whetstone H., Poon R., Wei Q., Keilty J., Proctor J., Wang L.W., Apte S.S., McGovern K., Alman B.A. and Wunder J.S. (2014). Hedgehog pathway inhibition in chondrosarcoma using the smoothened inhibitor IPI-926 directly inhibits sarcoma cell growth. Mol. Cancer Ther. 13, 1259-1269.

Cichon M.A., Degnim A.C., Visscher D.W. and Radisky D.C. (2010). Microenvironmental influences that drive progression from benign breast disease to invasive breast cancer. J. Mammary Gland Biol. Neoplasia 15, 389-397.

Cortez E., Roswall P. and Pietras K. (2014). Functional subsets of mesenchymal cell types in the tumor microenvironment. Semin. Cancer Biol. 25, 3-9.

Crawford Y., Kasman I., Yu L., Zhong C., Wu X., Modrusan Z., Kaminker J. and Ferrara N. (2009). PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. Cancer Cell 15, 21-34.

de Kruijf E.M., van Nes J.G., van de Velde C.J., Putter H., Smit V.T., Liefers G.J., Kuppen P.J., Tollenaar R.A. and Mesker W.E. (2011). Tumor-stroma ratio in the primary tumor is a prognostic factor in early breast cancer patients, especially in triple-negative carcinoma patients. Breast Cancer Res. Treat. 125, 687-696.

Del Casar J.M., Gonzalez L.O., Alvarez E., Junquera S., Marin L., Gonzalez L., Bongera M., Vazquez J. and Vizoso F.J. (2009). Comparative analysis and clinical value of the expression of metalloproteases and their inhibitors by intratumor stromal fibroblasts and those at the invasive front of breast carcinomas. Breast Cancer Res. Treat. 116, 39-52.

Desmedt C., Majjaj S., Kheddoumi N., Singhal S.K., Haibe-Kains B., El Ouriaghli F., Chaboteaux C., Michiels S., Lallemand F., Journe F., Duvillier H., Loi S., Quackenbush J., Dekoninck S., Blanpain C., Lagneaux L., Houhou N., Delorenzi M., Larsimont D., Piccart M. and Sotiriou C. (2012). Characterization and clinical evaluation of CD10+ stroma cells in the breast cancer microenvironment. Clin. Cancer Res. 18, 1004-1014.

Duda D.G., Duyverman A.M., Kohno M., Snuderl M., Steller E.J., Fukumura D. and Jain R.K. (2010). Malignant cells facilitate lung metastasis by bringing their own soil. Proc. Natl. Acad. Sci. USA 107, 21677-21682.

Dumont N., Liu B., Defilippis R.A., Chang H., Rabban J.T., Karnezis A.N., Tjoe J.A., Marx J., Parvin B. and Tlsty T.D. (2013). Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. Neoplasia 15, 249-262.

Fleming J.M., Miller T.C., Quinones M., Xiao Z., Xu X., Meyer M.J., Ginsburg E., Veenstra T.D. and Vonderhaar B.K. (2010). The normal breast microenvironment of premenopausal women differentially influences the behavior of breast cancer cells in vitro and in vivo. BMC Med. 8, 27.

Forsberg K., Valyi-Nagy I., Heldin C.H., Herlyn M. and Westermark B. (1993). Platelet-derived growth factor (PDGF) in oncogenesis: development of a vascular connective tissue stroma in xenotransplanted human melanoma producing PDGF-BB. Proc. Natl. Acad. Sci. USA 90, 393-397.

Galdiero M.R., Garlanda C., Jaillon S., Marone G. and Mantovani A. (2013). Tumor associated macrophages and neutrophils in tumor progression. J. Cell. Physiol. 228, 1404-1412.

Gold M.A., Brady W.E., Lankes H.A., Rose P.G., Kelley J.L., De Geest K., Crispens M.A., Resnick K.E. and Howell S.B. (2012). A phase II study of a urokinase-derived peptide (A6) in the treatment of persistent or recurrent epithelial ovarian, fallopian tube, or primary peritoneal carcinoma: a Gynecologic Oncology Group study. Gynecol. Oncol. 125, 635-639.

Gonda T.A., Varro A., Wang T.C. and Tycko B. (2010). Molecular biology of cancer-associated fibroblasts: can these cells be targeted in anti-cancer therapy? Semin. Cell Dev. Biol. 21, 2-10.

Hu M., Peluffo G., Chen H., Gelman R., Schnitt S. and Polyak K. (2009). Role of COX-2 in epithelial-stromal cell interactions and progression of ductal carcinoma in situ of the breast. Proc. Natl. Acad. Sci. USA 106, 3372-3377.

Hu M., Yao J., Carroll D.K., Weremowicz S., Chen H., Carrasco D., Richardson A., Violette S., Nikolskaya T., Nikolsky Y., Bauerlein E.L., Hahn W.C., Gelman R.S., Allred C., Bissell M.J., Schnitt S. and Polyak K. (2008). Regulation of in situ to invasive breast carcinoma transition. Cancer Cell 13, 394-406.

Huang M., Li Y., Zhang H. and Nan F. (2010). Breast cancer stromal fibroblasts promote the generation of CD44+CD24- cells through SDF-1/CXCR4 interaction. J. Exp. Clin. Cancer Res. 29, 80.

Hugo H.J., Lebret S., Tomaskovic-Crook E., Ahmed N., Blick T., Newgreen D.F., Thompson E.W. and Ackland M.L. (2012). Contribution of fibroblast and mast cell (afferent) and tumor (efferent) IL-6 effects within the tumor microenvironment. Cancer Microenviron. 5, 83-93

Ishihara A., Yoshida T., Tamaki H. and Sakakura T. (1995). Tenascin expression in cancer cells and stroma of human breast cancer and its prognostic significance. Clin. Cancer Res. 1, 1035-1041.

Iskaros B.F., Hu X., Sparano J.A. and Fineberg S.A. (1998). Tenascin pattern of expression and established prognostic factors in invasive breast carcinoma. J. Surg. Oncol. 68, 107-112.

Johnson L.M., Price D.K. and Figg W.D. (2013). Treatment-induced secretion of WNT16B promotes tumor growth and acquired resistance to chemotherapy: implications for potential use of inhibitors in cancer treatment. Cancer Biol. Ther. 14, 90-91.

Jotzu C., Alt E., Welte G., Li J., Hennessy B.T., Devarajan E., Krishnappa S., Pinilla S., Droll L. and Song Y.H. (2010). Adipose tissue-derived stem cells differentiate into carcinoma-associated fibroblast-like cells under the influence of tumor-derived factors.

Analyt. Cell. Pathol. (Amsterdam) 33, 61-79.

Karagiannis G.S., Poutahidis T., Erdman S.E., Kirsch R., Riddell R.H. and Diamandis E.P. (2012). Cancer-associated fibroblasts drive the progression of metastasis through both paracrine and mechanical pressure on cancer tissue. Mol. Cancer Res. 10, 1403-1418.

Kessenbrock K., Plaks V. and Werb Z. (2010). Matrix metalloproteinases: regulators of the tumor microenvironment. Cell 141, 52-67.

Kim K.J., Wang L., Su Y.C., Gillespie G.Y., Salhotra A., Lal B. and Laterra J. (2006). Systemic anti-hepatocyte growth factor monoclonal antibody therapy induces the regression of intracranial glioma xenografts. Clin. Cancer Res. 12, 1292-1298.

Kojima Y., Acar A., Eaton E.N., Mellody K.T., Scheel C., Ben-Porath I., Onder T.T., Wang Z.C., Richardson A.L., Weinberg R.A. and Orimo A. (2010). Autocrine TGF-beta and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. Proc. Natl. Acad. Sci. USA 107, 20009-20014.

Lee K.W., Yeo S.Y., Sung C.O. and Kim S.H. (2015). Twist1 is a key regulator of cancer-associated fibroblasts. Cancer Res. 75, 73-85.

Locatelli A., Lofgren K.A., Daniel A.R., Castro N.E. and Lange C.A. (2012). Mechanisms of HGF/Met signaling to Brk and Sam68 in breast cancer progression. Hormones Cancer 3, 14-25.

Loeffler M., Kruger J.A., Niethammer A.G. and Reisfeld R.A. (2006). Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. J. Clin. Invest. 116, 1955-1962.

Madar S., Goldstein I. and Rotter V. (2013). 'Cancer associated fibroblasts'--more than meets the eye. Trends Mol. Med. 19, 447-453.

Mani S.A., Guo W., Liao M.J., Eaton E.N., Ayyanan A., Zhou A.Y., Brooks M., Reinhard F., Zhang C.C., Shipitsin M., Campbell L.L., Polyak K., Brisken C., Yang J. and Weinberg R.A. (2008). The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133, 704-715.

Mao Y., Keller E.T., Garfield D.H., Shen K. and Wang J. (2013). Stromal cells in tumor microenvironment and breast cancer. Cancer Metastasis Rev. 32, 303-315.

Martinez-Outschoorn U.E., Balliet R.M., Rivadeneira D.B., Chiavarina B., Pavlides S., Wang C., Whitaker-Menezes D., Daumer K.M., Lin Z., Witkiewicz A.K., Flomenberg N., Howell A., Pestell R.G., Knudsen E.S., Sotgia F. and Lisanti M.P. (2010). Oxidative stress in cancer associated fibroblasts drives tumor-stroma co-evolution: A new paradigm for understanding tumor metabolism, the field effect and genomic instability in cancer cells. Cell Cycle 9, 3256-3276.

Martinez-Outschoorn U.E., Goldberg A., Lin Z., Ko Y.H., Flomenberg N., Wang C., Pavlides S., Pestell R.G., Howell A., Sotgia F. and Lisanti M.P. (2011). Anti-estrogen resistance in breast cancer is induced by the tumor microenvironment and can be overcome by inhibiting mitochondrial function in epithelial cancer cells. Cancer Biol. Ther. 12, 924-938.

Mercier I., Casimiro M.C., Wang C., Rosenberg A.L., Quong J., Minkeu A., Allen K.G., Danilo C., Sotgia F., Bonuccelli G., Jasmin J.F., Xu H., Bosco E., Aronow B., Witkiewicz A., Pestell R.G., Knudsen E.S. and Lisanti M.P. (2008). Human breast cancer-associated fibroblasts (CAFs) show caveolin-1 downregulation and RB tumor suppressor functional inactivation: Implications for the response to hormonal therapy. Cancer Biol. Ther. 7, 1212-1225.

Miki Y., Suzuki T., Tazawa C., Yamaguchi Y., Kitada K., Honma S.,

Moriya T., Hirakawa H., Evans D.B., Hayashi S., Ohuchi N. and Sasano H. (2007). Aromatase localization in human breast cancer tissues: possible interactions between intratumoral stromal and parenchymal cells. Cancer Res. 67, 3945-3954.

Molina J.R., Reid J.M., Erlichman C., Sloan J.A., Furth A., Safgren S.L., Lathia C.D. and Alberts S.R. (2005). A phase I and pharmacokinetic study of the selective, non-peptidic inhibitor of matrix metalloproteinase BAY 12-9566 in combination with etoposide and carboplatin. Anticancer Drugs 16, 997-1002.

Moskovits N., Kalinkovich A., Bar J., Lapidot T. and Oren M. (2006). p53 Attenuates cancer cell migration and invasion through repression of SDF-1/CXCL12 expression in stromal fibroblasts. Cancer Res. 66, 10671-10676.

Mueller K.L., Madden J.M., Zoratti G.L., Kuperwasser C., List K. and Boerner J.L. (2012). Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. Breast Cancer Res. 14, R104.

Nguyen D.H., Oketch-Rabah H.A., Illa-Bochaca I., Geyer F.C., Reis-Filho J.S., Mao J.H., Ravani S.A., Zavadil J., Borowsky A.D., Jerry D.J., Dunphy K.A., Seo J.H., Haslam S., Medina D. and Barcellos-Hoff M.H. (2011). Radiation acts on the microenvironment to affect breast carcinogenesis by distinct mechanisms that decrease cancer latency and affect tumor type. Cancer Cell 19, 640-651.

Niemiec J.A., Adamczyk A., Ambicka A., Mucha-Malecka A., W M.W. and Rys J. (2014). Triple-negative, basal marker-expressing, and high-grade breast carcinomas are characterized by high lymphatic vessel density and the expression of podoplanin in stromal fibroblasts. Appl. Immunohistochem. Mol. Morphol. 22, 10-16.

O'Connell J.T., Sugimoto H., Cooke V.G., MacDonald B.A., Mehta A.I., LeBleu V.S., Dewar R., Rocha R.M., Brentani R.R., Resnick M.B., Neilson E.G., Zeisberg M. and Kalluri R. (2011). VEGF-A and Tenascin-C produced by S100A4+ stromal cells are important for metastatic colonization. Proc. Natl. Acad. Sci. USA 108, 16002-16007.

Orimo A., Gupta P.B., Sgroi D.C., Arenzana-Seisdedos F., Delaunay T., Naeem R., Carey V.J., Richardson A.L. and Weinberg R.A. (2005). Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121, 335-348.

Park J.E., Lenter M.C., Zimmermann R.N., Garin-Chesa P., Old L.J. and Rettig W.J. (1999). Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J. Biol. Chem. 274, 36505-36512.

Park S.Y., Kim H.M. and Koo J.S. (2015). Differential expression of cancer-associated fibroblast-related proteins according to molecular subtype and stromal histology in breast cancer. Breast Cancer Res. Treat. 149, 727-741.

Pavlides S., Tsirigos A., Vera I., Flomenberg N., Frank P.G., Casimiro M.C., Wang C., Fortina P., Addya S., Pestell R.G., Martinez-Outschoorn U.E., Sotgia F. and Lisanti M.P. (2010). Loss of stromal caveolin-1 leads to oxidative stress, mimics hypoxia and drives inflammation in the tumor microenvironment, conferring the "reverse Warburg effect": a transcriptional informatics analysis with validation. Cell Cycle 9, 2201-2219.

Pavlides S., Whitaker-Menezes D., Castello-Cros R., Flomenberg N., Witkiewicz A.K., Frank P.G., Casimiro M.C., Wang C., Fortina P., Addya S., Pestell R.G., Martinez-Outschoorn U.E., Sotgia F. and Lisanti M.P. (2009). The reverse Warburg effect: aerobic glycolysis

in cancer associated fibroblasts and the tumor stroma. Cell Cycle 8, 3984-4001.

Petersen O.W., Nielsen H.L., Gudjonsson T., Villadsen R., Rank F., Niebuhr E., Bissell M.J. and Ronnov-Jessen L. (2003). Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am. J. Pathol. 162, 391-402.

Pontiggia O., Sampayo R., Raffo D., Motter A., Xu R., Bissell M.J., Joffe E.B. and Simian M. (2012). The tumor microenvironment modulates tamoxifen resistance in breast cancer: a role for soluble stromal factors and fibronectin through beta1 integrin. Breast Cancer Res. Treat. 133, 459-471.

Przybylo J.A. and Radisky D.C. (2007). Matrix metalloproteinase-induced epithelial-mesenchymal transition: tumor progression at Snail's pace. Int. J. Biochem. Cell Biol. 39, 1082-1088.

Qian B.Z., Li J., Zhang H., Kitamura T., Zhang J., Campion L.R., Kaiser E.A., Snyder L.A. and Pollard J.W. (2011a). CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 475, 222-225.

Qian N., Ueno T., Kawaguchi-Sakita N., Kawashima M., Yoshida N., Mikami Y., Wakasa T., Shintaku M., Tsuyuki S., Inamoto T. and Toi M. (2011b). Prognostic significance of tumor/stromal caveolin-1 expression in breast cancer patients. Cancer Sci. 102, 1590-1596.

Ranogajec I., Jakic-Razumovic J., Puzovic V. and Gabrilovac J. (2012). Prognostic value of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9) and aminopeptidase N/CD13 in breast cancer patients. Med. Oncol. 29, 561-569.

Raz Y. and Erez N. (2013). An inflammatory vicious cycle: Fibroblasts and immune cell recruitment in cancer. Exp. Cell Res. 319, 1596-1603.

Roy R., Yang J. and Moses M.A. (2009). Matrix metalloproteinases as novel biomarkers and potential therapeutic targets in human cancer. J. Clin. Oncol. 27, 5287-5297.

Sarchio S.N., Scolyer R.A., Beaugie C., McDonald D., Marsh-Wakefield F., Halliday G.M. and Byrne S.N. (2014). Pharmacologically antagonizing the CXCR4-CXCL12 chemokine pathway with AMD3100 inhibits sunlight-induced skin cancer. J. Invest. Dermatol. 134, 1091-1100.

Sasano H., Miki Y., Shibuya R. and Suzuki T. (2010). Aromatase and in situ estrogen production in DCIS (ductal carcinoma in situ) of human breast. J. Steroid Biochem. Mol. Biol. 118, 242-245.

Schoppmann S.F., Berghoff A., Dinhof C., Jakesz R., Gnant M., Dubsky P., Jesch B., Heinzl H. and Birner P. (2012). Podoplanin-expressing cancer-associated fibroblasts are associated with poor prognosis in invasive breast cancer. Breast Cancer Res. Treat. 134, 237-244.

Scott A.M., Wiseman G., Welt S., Adjei A., Lee F.T., Hopkins W., Divgi C.R., Hanson L.H., Mitchell P., Gansen D.N., Larson S.M., Ingle J.N., Hoffman E.W., Tanswell P., Ritter G., Cohen L.S., Bette P., Arvay L., Amelsberg A., Vlock D., Rettig W.J. and Old L.J. (2003). A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin. Cancer Res. 9, 1639-1647.

Shao Z.M., Nguyen M. and Barsky S.H. (2000). Human breast carcinoma desmoplasia is PDGF initiated. Oncogene 19, 4337-4345.

Shekhar M.P., Santner S., Carolin K.A. and Tait L. (2007). Direct involvement of breast tumor fibroblasts in the modulation of tamoxifen sensitivity. Am. J. Pathol. 170, 1546-1560.

Soon P.S., Kim E., Pon C.K., Gill A.J., Moore K., Spillane A.J., Benn D.E. and Baxter R.C. (2013). Breast cancer-associated fibroblasts

induce epithelial-to-mesenchymal transition in breast cancer cells. Endocr. Relat. Cancer 20, 1-12.

Spenle C., Gasser I., Saupe F., Janssen K.P., Arnold C., Klein A., van der Heyden M., Mutterer J., Neuville-Mechine A., Chenard M.P., Guenot D., Esposito I., Slotta-Huspenina J., Ambartsumian N., Simon-Assmann P. and Orend G. (2015). Spatial organization of the tenascin-C microenvironment in experimental and human cancer. Cell Adh. Migr. 9, 4-13.

Strutz F., Zeisberg M., Hemmerlein B., Sattler B., Hummel K., Becker V. and Muller G.A. (2000). Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. Kidney Int. 57, 1521-1538.

Tada Y., Hiroshima K., Shimada H., Morishita N., Shirakawa T., Matsumoto K., Shingyoji M., Sekine I., Tatsumi K. and Tagawa M. (2015). A clinical protocol to inhibit the HGF/c-Met pathway for malignant mesothelioma with an intrapleural injection of adenoviruses expressing the NK4 gene. SpringerPlus 4, 358.

Tan W., Zhang W., Strasner A., Grivennikov S., Cheng J.Q., Hoffman R.M. and Karin M. (2011). Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature 470, 548-553.

Tchou J. and Conejo-Garcia J. (2012). Targeting the tumor stroma as a novel treatment strategy for breast cancer: shifting from the neoplastic cell-centric to a stroma-centric paradigm. Adv. Pharmacol. 65, 45-61.

Thottassery J.V., Sambandam V., Allan P.W., Maddry J.A., Maxuitenko Y.Y., Tiwari K., Hollingshead M. and Parker W.B. (2014). Novel DNA methyltransferase-1 (DNMT1) depleting anticancer nucleosides, 4'-thio-2'-deoxycytidine and 5-aza-4'-thio-2'-deoxycytidine. Cancer Chemother. Pharmacol. 74, 291-302.

Tlsty T.D. (2001). Stromal cells can contribute oncogenic signals. Semin. Cancer Biol. 11, 97-104.

Trimboli A.J., Cantemir-Stone C.Z., Li F., Wallace J.A., Merchant A., Creasap N., Thompson J.C., Caserta E., Wang H., Chong J.L., Naidu S., Wei G., Sharma S.M., Stephens J.A., Fernandez S.A., Gurcan M.N., Weinstein M.B., Barsky S.H., Yee L., Rosol T.J., Stromberg P.C., Robinson M.L., Pepin F., Hallett M., Park M., Ostrowski M.C. and Leone G. (2009). Pten in stromal fibroblasts suppresses mammary epithelial tumours. Nature 461, 1084-1091.

Trimis G., Chatzistamou I., Politi K., Kiaris H. and Papavassiliou A.G. (2008). Expression of p21waf1/Cip1 in stromal fibroblasts of primary breast tumors. Hum. Mol. Genet. 17, 3596-3600.

Trimmer C., Sotgia F., Whitaker-Menezes D., Balliet R.M., Eaton G., Martinez-Outschoorn U.E., Pavlides S., Howell A., Iozzo R.V., Pestell R.G., Scherer P.E., Capozza F. and Lisanti M.P. (2011). Caveolin-1 and mitochondrial SOD2 (MnSOD) function as tumor suppressors in the stromal microenvironment: a new genetically tractable model for human cancer associated fibroblasts. Cancer Biol. Ther. 11, 383-394.

Tyan S.W., Kuo W.H., Huang C.K., Pan C.C., Shew J.Y., Chang K.J., Lee E.Y. and Lee W.H. (2011). Breast cancer cells induce cancer-associated fibroblasts to secrete hepatocyte growth factor to enhance breast tumorigenesis. PLoS One 6, e15313.

Warburg O. (1956). On the origin of cancer cells. Science 123, 309-314.

Weigel M.T., Banerjee S., Arnedos M., Salter J., A'Hern R., Dowsett M. and Martin L.A. (2013). Enhanced expression of the PDGFR/Abl signaling pathway in aromatase inhibitor-resistant breast cancer. Ann. Oncol. 24, 126-133.

Witkiewicz A.K., Dasgupta A., Sammons S., Er O., Potoczek M.B.,

Guiles F., Sotgia F., Brody J.R., Mitchell E.P. and Lisanti M.P. (2010). Loss of stromal caveolin-1 expression predicts poor clinical outcome in triple negative and basal-like breast cancers. Cancer Biol. Ther. 10, 135-143.

Witkiewicz A.K., Dasgupta A., Sotgia F., Mercier I., Pestell R.G., Sabel M., Kleer C.G., Brody J.R. and Lisanti M.P. (2009). An absence of stromal caveolin-1 expression predicts early tumor recurrence and poor clinical outcome in human breast cancers. Am. J. Pathol. 174, 2023-2034.

Yamaguchi Y., Takei H., Suemasu K., Kobayashi Y., Kurosumi M., Harada N. and Hayashi S. (2005). Tumor-stromal interaction through the estrogen-signaling pathway in human breast cancer. Cancer Res. 65, 4653-4662.

Yamashita M., Ogawa T., Zhang X., Hanamura N., Kashikura Y., Takamura M., Yoneda M. and Shiraishi T. (2012). Role of stromal myofibroblasts in invasive breast cancer: stromal expression of alpha-smooth muscle actin correlates with worse clinical outcome.

Breast Cancer 19, 170-176.

Yazhou C., Wenlv S., Weidong Z. and Licun W. (2004). Clinico-pathological significance of stromal myofibroblasts in invasive ductal carcinoma of the breast. Tumour Biol. 25, 290-295.

Zeisberg E.M., Potenta S., Xie L., Zeisberg M. and Kalluri R. (2007). Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. Cancer Res. 67, 10123-10128.

Zhang B., Zhang Y., Bowerman N.A., Schietinger A., Fu Y.X., Kranz D.M., Rowley D.A. and Schreiber H. (2008). Equilibrium between host and cancer caused by effector T cells killing tumor stroma. Cancer Res. 68, 1563-1571.

Zhu Y., Cheng M., Yang Z., Zeng C.Y., Chen J., Xie Y., Luo S.W., Zhang K.H., Zhou S.F. and Lu N.H. (2014). Mesenchymal stem cell-based NK4 gene therapy in nude mice bearing gastric cancer xenografts. Drug Design Dev. Ther. 8, 2449-2462.