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^{PDGFRα}, and CAF^{PDGFRβ} 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^{PDGFRα} phenotype is associated with angiogenesis and macrophage recruitment, and the CAF^{PDGFRβ} 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 (Trimits et al., 2008), PTEN (Trimbolli et al., 2009), caveolin-1 (Trimmer et al., 2011), and p16INK4A (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 (Trimbolli et al., 2009; Cichon et al., 2010; Nguyen et al., 2011). In addition, inoculation of breast cancer cells (MCF-7 and

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

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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 secrete 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 (Przybyle 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 promote 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-

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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)-1α and NF-κB 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.

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**Conflicts of interest.** None.

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