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The cancer-associated fibroblasts and drug resistance

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Abstract. – The present treatment of solid tumors is plagued by drug resistance. Despite continued development of meticulously designed therapeutic scheme, cancer cells remain poorly being completely eliminated. Because therapeutic resistance is a problem with the drug used in cancer therapy, most of the studies about the drugs resistance have focused on the role of epithelial cancer cells themselves. However, it is becoming increasingly apparent that tumor microenvironment could provide a shelter for tumor cells which keep their survival after initial drug exposure. Cancer-associated fibroblasts (CAFs) are the crucial component of the tumor microenvironment; substantial evidence suggests that the CAFs mediate resistance of solid tumor cells to the anticancer drugs. In this review, we describe how the CAFs may be involved in the resistance of tumor cells to the therapeutic agents and present some of the emerging therapeutic targets for modulation this resistant phenotype.

Key Words:
Cancer-associated fibroblasts, Drug resistance, Signaling pathways.

Introduction

Wealth of data suggests that drug resistance remains a major obstacle to successful eliminate the tumor cells. Many studies have focused on tumor cell autonomous mechanisms which led to drug resistance. Indeed, cancer susceptibility vs. resistance is partly determined by the dynamic interplay between tumor cells and their microenvironment. The proposed molecular mechanisms responsible for resistance to current treatments mainly focused on epithelial cancer cells themselves. The cancer cell can employ multiple strategies favor themselves survival following initial drug exposure. These mechanisms include[1]: (1) decreased intracellular concentration of the drug via over-expression of drug transporters; (2) altered the drug target via point mutations or over-expression of the target gene; (3) improved the ability to repair DNA damage; (4) alterations in cell cycle checkpoint mediators; (5) activation of anti-apoptotic signaling pathways. It is also known that cancer cells do not exist in isolation in tumor tissues, but rather intimately interact with non-neoplastic host cells[2]. It is becoming increasingly apparent that tumor microenvironment not only provide a shelter for tumor cells that allow their survival after initial drug exposure[3,4], but also serves as a paracrine niche for cancer cells to promote them growth and metastasis.

The stromal fibroblasts were activated in response to the cancer cells, the activated fibroblasts called cancer-associated fibroblasts (CAFs). CAFs are the predominant stromal cells in the tumor tissue and can promote cancer cell growth, invasiveness and angiogenesis mainly through secretion of soluble factors, such as growth factors or cytokines[5,6]. Mounting evidence suggests that the CAFs play a crucial role in drug resistance[7-9]. Compared to tumor cells, CAFs are relative genetic stability with lesser probability to develop the drug-resistance and represent an attractive therapeutic target with less risk of drug resistance and tumor recurrence[10]. In this review, we discuss the several crucial signaling pathways between the tumor cells and CAFs, which led to resistance of tumor cells to therapeutic agents. We then highlight the current advances in the development of novel therapeutic strategies against CAFs alone or in combination with conventional therapies.

Cancer-Associated Fibroblasts

Cancer-associated fibroblasts (CAFs) are the principal component of the tumor microenvironment and originate from heterogeneous cell types[11,12]. The CAFs modulate the behavior of

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adjacent cancer cells by secreting various growth factors, cytokines and extracellular matrix[5,11,12]. Increasing evidence indicated that CAFs-mediated resistance phenomenon is more common. CAFs can affect the sensitivity of pancreatic carcinoma cells to chemo- or radio-therapy by the release of soluble factors, and led to tumor cells be less sensitive to gemcitabine[8]. Elimination of CAFs *in vivo* by a DNA vaccine targeted to fibroblast activation protein can improve the effects of doxorubicin[13]. CAFs also can protect neck squamous cell carcinoma cells from cetuximab treatment by secreting MMPs[7], and protect PC3 cells from sorafenib-induced cell death by the up-regulation of BCL- XL[14]. All these data suggested that CAFs are a decisive player in therapeutic resistance[15,16].

Cancer Stem Cells

With increasing evidence supporting the existence of cancer stem cells (CSCs) or the cancer-initiating cell (CIC), CSCs are considered to be a unique subpopulation in tumors, which possess self-renewal ability and to differentiate into cancer cells. The expression of the embryonic stem cell marker POU5F1 has been shown in bladder cancer samples[17]. Treatment with chemotherapy increased the percentage of $CD44^{+}/CD24^{-/low}$ cells in breast cancer[18], and $CD44^{+}/CD24^{-/low}$ is the stem cell marker of breast cancer[19]. The stem cell-like properties play crucial roles in tumor initiation, progression, and therapeutic refractoriness[20]. Substantial evidence suggests that the CSCs are inherently resistant to radio-chemotherapy[21] owing to still poorly understood mechanisms that include: (1) Like normal pluripotent stem cells, CSCs entry into long-term dormant state[22]; (2) CSCs are generally more quiescent, and have enhanced DNA repair capacity[23]; (3) CSCs can be difficult to reach because they can reside in a particular niche in the tumor tissue, and have the ability to self-renew[24]; (4) Moreover, CSCs express ABC transporters, ABCG2 and ABCB1 (also known as MDR1), lead to multidrug resistance[25,26]. All these studies suggest that CSCs has the capacity to repopulate tumors and drive malignant progression and mediate drug resistance.

It is becoming increasingly clear that CSCs do not exist in isolation, but rather reside in a special stromal niche. CAFs serve as paracrine niche for supporting the stemness of CSCs has been proved[27,28]. Study[29] indicated that CAFs from castration-resistant prostate cancer (CRPC) are far more potent than their androgen-dependent prostate cancer (ADPC) counterparts to support the homologous origin of CSC. This suggests that the CAFs derived from CRPC secrete stronger signaling molecule(s) than the counterparts derived from ADPC. The CAFs in avascular area of the tumor microenvironment strongly expressed the transmembrane glycoprotein CD44, compared to their counterparts. CD44-positive CAFs maintain the stemness of CSCs/CICs via direct interactions, and the CD44 also was involved in cancer drug resistance[28]. The CAFs also significantly increased the expression of IL-17A in response to chemotherapy in colorectal tumor tissue; the majority of CICs expressed IL-17A receptor. So the IL-17A which derived from the CAFs bond to IL-17A receptor on CICs, and led to the CICs maintenance and CICs therapeutic resistance through up-regulation of NF-Kb[30].

There is a growing body of data[31,32] indicated that the residual tumor cell populations surviving after conventional treatment may be enriched the subpopulations of cells with both tumor-initiating and mesenchymal features. Consistent with this, recent studies[33,34] have suggested that chemotherapy can lead to resistance and induction of the epithelial to mesenchymal transition (EMT) in cancer cells, and lead to the acquisition of stem cell properties[35]. In this context, EMT may comprise epigenetic and/or a genetic change resulting in altered gene expressions. The CAFs also can induce EMT in epithelial cancer cells[36,37]. Based on these studies we can deduce that CAFs can induce epithelial cancer cells switch to a motile, mesenchymal phenotype, and render cancer cells with cancer stem cells properties. Study[38] also indicated that the conditioned medium (CM) from CAFs can induce EMT in NSCLC cells, and these cells also acquired characteristics of stem cell-like qualities. Anx A1 which secreted by CAFs can regulate EMT in cE1 cells, and these cells which go though EMT can lead to CSC generation[27]. In line with this, increasing evidence suggests that CAFs can secrete TGF-β, and TGF-β signaling mediate EMT and maintenance the stemness of differential type of cancer cells which derived from epithelial [39,40] via TGF-β/Smad signalling pathway[41], or TGF-β-FOXO pathway[40]. There is a consensus[41] that TGF-β is a strong inducer of EMT in epithelial cells.

The carbonic anhydrase IX (CA IX), a transmembrane enzyme, contributes to acidification of the extracellular microenvironment[42,43], and

the acidic microenvironment induce the tumor cells to acquire the metastatic phenotypes and chemo-resistance to basic anticancer drugs. The CAFs can express CA IX, and leads to extracellular acidification, in turn enhancing MMP-2 and-9 activation and leading to EMT in PCa cells[44]. The CAFs also can induce CCL2 production through STAT3 activation, and the CAFs-derived CCL2, in turn, promotes cancer progression by regulating CSCs through activation of the NOTCH pathway[27]. NOTCH activation has been shown to promote the self-renewal of mammary stem cells[45].

All these studies indicated that CAFs can render cancer cells with cancer stem cells properties through EMT, and maintain the stemness via direct interactions, and lead to drug resistance.

PDGF Signaling

Platelet-derived growth factor (PDGF) is a dimeric protein, and exert its biological effects by binding to two structurally related tyrosine kinase receptors, PDGF-α and β receptor[46]. PDGF are potent mitogens, survival factors, and chemoattractants for many mesenchymal cells. Upon ligand binding, PDGF receptors homo- or heterodimerize and phosphorylate the specific tyrosine residues, initiating signaling cascades that lead to growth, actin cytoskeleton rearrangements, and chemotaxis[47].

The interstitial fluid pressure is high in the center of solid tumors and low in the periphery and surrounding tissue[48,49]. The interstitial hypertension contributes to the poor delivery of therapeutic agents to tumor cells[50]. One important cause of drug resistance is the limited ability of drugs to penetrate into tumor tissue and to reach the tumor cells in a potentially lethal concentration. The feasible way to improve the distribution of therapeutic agents in the tumor tissue reduce the high interstitial fluid pressure (IFP)[51,52]. A number of studies have shown that PDGF/PDGF-R signaling pathway plays a critical role in the IFP[51,53]; inhibition of PDGF-R signaling can decrease IFP and, hence, enhance the effects chemotherapeutic reagents[49,54].

Most cancer cells which secrete PDGF ligands, do not express PDGF-Rs, suggesting that PDGF may act in a paracrine manner[55,56]. Studies[49,51] indicated that expression of PDGF receptor is restricted to the tumor stroma. Fibroblasts contribute to the high IFP observed in solid tumors; one of mechanism which lead to high IFP via PDGF-R signaling pathway[57,58]. In agreement with these findings, the CAFs expressed the PDGFα and ß-receptors[15,57]. Targeting PDGF receptors expressed on fibroblasts with a combination of imatinib mesylate (Glivec) and chemotherapeutic agent can lower the tumor interstitial hypertension, and enhance tumor uptake and therapeutic effects of anticancer drugs[49,58]. In line with this, it has been shown that PDGF-R tyrosine kinase inhibitor nilotinib did not suppress tumor growth but significantly decreased stromal reactivity; in contrast, treatment with mTOR inhibitor everolimus decreased tumor growth, but no effect on the stromal reactivity. They have a synergistic effect when nilotinib and everolimus were administered in combination, and the tumor growth, tumor incidence and lymph node metastasis were much more inhibited with the combination treatment[59]. In addition, tumor cells refractory to anti-VEGF therapy can stimulate adjacent CAFs to secrete proangiogenic platelet-derived growth factor C (PDGF-C), which compensates for the neutralization of VEGF and promotes tumor angiogenesis[15]. Moreover, CAFs suppress blood vessel formation leading to the sparse vasculature, making drug delivery more difficult[60].

HGF/MET Signaling

MET (c-Met) is a heterodimeric transmembrane receptor tyrosine kinase and present on most types‘ cell. The sole identified ligand for MET is HGF. Binding of HGF to MET triggers multiple activities in the cell, including motility, proliferation, survival, and morphogenesis[61]. Tumor cells of epithelial origin frequently over-express c-Met, resulting in increased responsiveness to HGF produced by stromal cells[62]; and HGF is mainly secreted by CAFs[63,9]. Amplification and/or over-expression of MET and/or HGF was correlate with poor clinical prognosis in patients with solid tumors[64,65], and confer acquired resistance to targeted therapy agents[66,67].

A growing body of evidence suggests that the multiple tyrosine kinase inhibitors (TKIs), which targeted oncogenic receptor kinases (RTKs) of tumor cells, do not result in a measurable and sustainable clinical benefit in a vast majority of tumors. This emphasizes the need for further investigations into their resistance mechanisms against TKIs. Two downstream survival signalling pathways commonly engaged by RTKs: the phosphatidylinositol-3-OH kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathways. Consequently, an in-

crease in RTK-ligand levels, which produced by tumor cells or tumor stroma could confer resistance to inhibitors of an oncogenic kinase with a similar signaling output[68]. The HGF confers resistance to the BRAF inhibitor in BRAF-mutant melanoma cells; HGF also promotes lapatinib resistance in HER2-amplified breast cancer cell lines[68,69]. In line with this, the cancer cells with epidermal growth factor receptor (EGFR)-activating mutations show good clinical response to selective tyrosine kinase inhibitors (TKI) which targeting EGFR in the early stages of drug treatment, but these cancer cells can recruit fibroblasts, which produce HGF; the HGF, as a paracrine mediator, induces EGFR-TKI resistance by restoring PI3K/Akt pathway via MET oncogene, but not EGFR or ErbB3[66]. Taken together, these studies indicate that the presence of stromal HGF confers resistance to therapy. In addition, the CAFs secreted HGF can activate the MET which expressed on the cancer-initiating cells (CICs) via paracrine circuit in colon cancer; and sustain distinctive CIC properties such as long-term self-renewal, and lead to anti-EGFR therapy[63]

CXCR4 Pathway

CXCR4 is one of chemokine receptors, and most widely expressed by malignant tumors, and its role in tumor biology is most thoroughly studied. The chemokine CXCL12 (stromal cell-derived factor-1, SDF-1) is the sole ligand of CXCR4[70]. In the tumor microenvironment, the autocrine and paracrine chemokine/chemokine receptor loops interact to promote tumor cell survival and growth[70]. Study indicated that SDF-1/CXCR4 signaling contributes to chemoresistance by expressing anti-apoptotic proteins such as BCL-2, BCL-XL[71,72].

CAFs are an important source of CXCL12 in the tumor niche. Soluble factor CXCL12 released by stromal fibroblasts attracted the prostate cancer cells which express CXCR4 to the stromal microenvironment, and kept their survival after initial drug exposure. Disrupt CXCR4/CXCL12 signaling sensitized prostate cancer cells to docetaxel[73]. This result is supported by the study that CXCR4-positive tumor cells homed to the CXCL12-rich bone marrow niche[74]. In addition, the fibroblast-derived CXCL12 also can promote cancer progression by regulating CSCs through activation of the NOTCH pathway[27]. In agreement with this study that NOTCH pathway activation has been shown to promote the self-renewal of mammary stem cells[45]. Taken together, these data provide supporting evidence that SDF1/CXCL12 involved in *de novo* acquired drug resistance and represent alternative targets for cancer therapy[75,76].

IL-6 Signaling

Emerging evidence suggests that the dynamic interaction between tumor cells and stroma fibroblasts during development is reciprocal. Tumor cells produce high level of IL-6; the IL-6 activated the neighboring fibroblasts, which in turn secrete matrix metalloproteinase MMP-2 and MMP-9 (MMP) which elicit EMT and promotes the generation of cancer stem cells[37]. In addition, the tumor cells, which fail to produce autocrine IL-6, respond to paracrine IL-6 signal which come from CAFs, markedly. IL-6 promoted growth and invasion of cancer cells through the chronic activation of STAT3[77]. In line with this, IL-6-induced STAT3 protects myeloma cells from Fas-mediated apoptosis and express high levels of the anti-apoptotic protein BCL-XL[78]. The study indicated that NSCLC cells expressing mutant EGFR are dependent on the IL-6 axis for their long-term proliferation/survival[79], and lead to primary and acquired erlotinib resistance[32]. All these indicated that the IL-6 created a "chemo-resistant niche" that promotes the survival of a minimal residual tumor burden and serves as a reservoir for eventual tumor relapse[80].

Conclusions

Despite meticulously designed therapeutic agents, drug resistance remains a vexing problem in the treatment of cancer patients; at present, many studies have focused on tumor-cell autonomous mechanisms of resistance. To develop more efficient therapies for prevention of resistance, it is important to further figure out the mechanisms that cause current therapies to fail. In this review, we discussed the important of CAFs in drug resistance; and the main mechanisms which mediate the resistance of solid tumor cells to therapeutic agents (Figure 1). More and more studies suggested that CAFs play decisive roles in the drug resistance. Thereby, the effective cancer therapies lie in a combination of therapies that target not only the bulk of the tumor but also the CAFs.

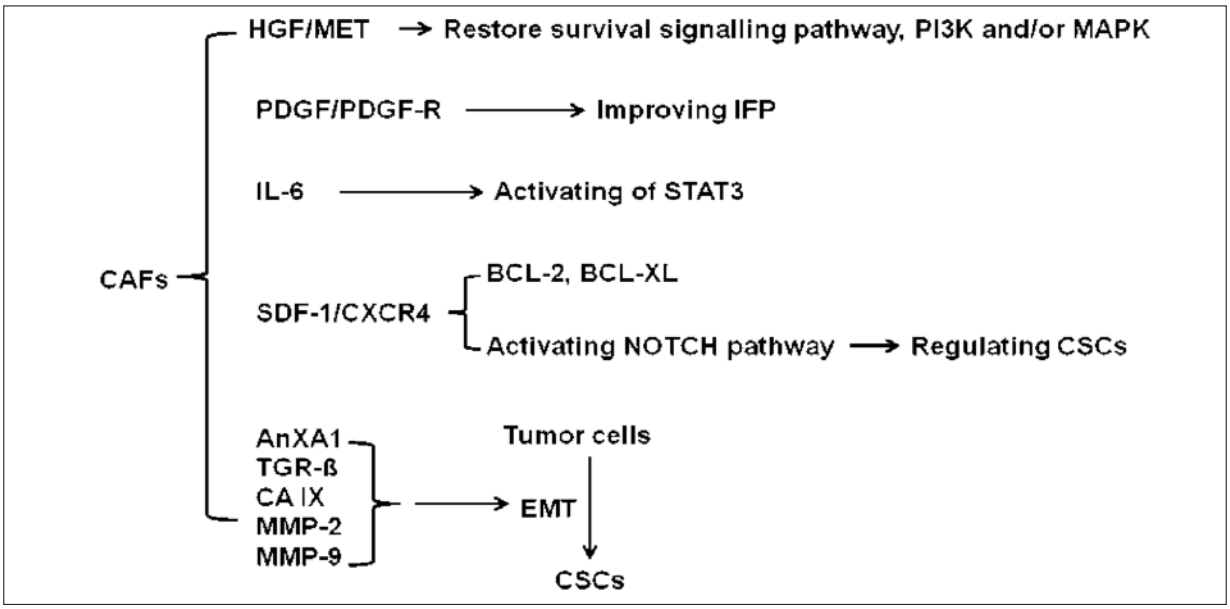

Figure 1. Several currently mechanisms of cancer-associated fibroblasts (CAFs) lead to therapeutic resistance. This diagram displays that CAFs play one important role in drug resistance via paracrine manner.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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