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Chapter 3

Tumor Fibroblasts: Pivotal Players in Drug Resistance

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Abstract

Chemotherapy remains one of the mainstay options for treating cancer. The dawn of the post-genomic era has witnessed several major breakthroughs in personalized medicine, and it has been an exciting time for the emergence of targeted chemotherapy for cancers with distinctive molecular characteristics. Yet, the development of drug resistance continues to hinder the success of chemotherapy, and it is often viewed as the outcome of cancer cell-autonomous processes. In fact, recent evidence points to the fact that the host environment itself can be equally guilty. Tumor fibroblasts - one of the responsible perpetrators - cooperate with the tumor cells to initiate and drive tumor progression. These cells

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are prominent members in most carcinoma tissues, and they provide a protective niche for tumor cells to escape from cytotoxic effects of chemotherapy. This chapter discusses the different types and origins of fibroblast population that exist within solid tumors, and their roles in dictating the response of tumors to therapies. The underlying key mechanisms by which the dynamic tumor-fibroblast cell interaction allow escape from therapeutic drugs and the eventual development of drug resistance are also discussed. This evidence supports the notion that tumor fibroblasts can potentially be a good target in overcoming drug resistance, and such tumor-fibroblast cell interaction should be targeted for designing more effective and less resistance-prone anti-cancer therapies.

Keywords: Tumor microenvironment, fibroblast, myofibroblasts, extracellular matrix remodeling, epithelial-mesenchymal transition, chemoresistance

Challenges in Cancer Chemotherapy

Chemotherapy remains one of the important therapeutic options for most patients with cancer. This field has grown tremendously over the past years, from "one drug-fits-all" strategy to targeted therapy using small molecule inhibitors that focus on "addicted" oncogenic pathways utilized by the tumor cells. Encouraging clinical results were observed with the use of epidermal growth factor (EGF) receptor inhibitors in non-small cell lung cancer [1], Bcr-Abl inhibitors in chronic myeloid leukemia [2] and c-KIT inhibitors in gastrointestinal stromal tumors [3]. Yet, the efficacy of chemotherapy, whether the conventional cytotoxic drugs or targeted small molecule inhibitors, in a single-agent setting or in drug combination, is severely hampered by the emergence of drug resistance [4, 5]. Drug resistance can be classified into two categories: intrinsic (also known as innate or *de novo*) resistance and acquired (or extrinsic) resistance. Patients with intrinsic drug resistance do not respond to the therapy from the start of treatment. In contrast, patients with acquired resistance responded initially to the treatment, but continued treatment ultimately leads to tumor recurrence and metastasis [6].

Development of drug resistance is often thought as the outcome of cancer cell-autonomous processes. Many studies elegantly demonstrate the acquisition of drug resistance through expression of ATP-binding cassette receptors which pump out drugs from the cancer cells [7]. Others suggest that the increased drug metabolism in cancer cells leads to increased detoxification

and reduced cytotoxic activities [8]. Cancer cells can also strengthen their defense signaling pathways to overcome drug-induced apoptosis [8]. All these processes are partly attributable to the development of acquired drug resistance, which require exposure to chemotherapeutic drugs.

The factors contributing to the development of innate drug resistance are less clear; however, accumulating evidence is pointing to the tumor microenvironment as a major player. Interaction of cancer cells with the surrounding extracellular matrix, via cell surface integrins, dramatically improved the survival of cancer cells at both primary and secondary sites, and when exposed to chemotherapeutic drugs [9]. Recently, the fibroblast population within the tumor microenvironment has emerged as another key player in contributing to the drug-resistant phenotype of cancer cells. This is especially apparent in tumors with evident desmoplastic reaction such as pancreatic cancer, which is well-known for its lethality due to limited response to conventional chemotherapy [10]. An understanding of the molecular mechanisms underlying fibroblasts-mediated drug resistance might help in devising novel strategies to overcome such resistance. This review summarizes the significance of several key mechanisms associated with fibroblasts-mediated drug resistance in cancer therapy.

Types and Origins of Tumor Fibroblasts

To date, it is unclear how many types of tumor fibroblasts exist within the tumor microenvironment. This is probably due to the lack of specific markers which can identify different subtypes of this cell population [11]. Tumor fibroblasts in general share similar phenotypes with myofibroblasts– the type of fibroblasts that are activated during wound healing [12]. These fibroblasts are thought to derive from resident fibroblasts undergoing transdifferentiation after stimulation by tumor secreted growth factors, which probably mimic the trigger of a wound healing-like process [13]. Unlike resident or benign fibroblasts, tumor fibroblasts show increased expression of fibronectin, fibroblast activation protein (FAP), tenascin-C, desmin and alpha-smooth muscle actin (α-SMA) [11, 14]. These fibroblasts are responsible for synthesizing and depositing various growth factors and extracellular matrix (ECM) proteins, including matrix metalloproteinases (MMPs), fibrin, heparanase and collagen, which are important for extracellular remodeling [11, 13].

Growing evidence is now indicating that these tumor fibroblasts may be transformed by the tumor cells. For example, *TP53* and *PTEN* somatic mutations were observed in the fibroblast within the breast carcinoma tissues [15]. These mutations are well-known for their roles in tumor progression and response to chemotherapy if found expressed in the tumor cells. Indeed, high frequency of stromal *TP53* mutation is associated with lymph node metastases in sporadic breast cancers [16], and loss of *PTEN* function in mouse mammary stroma accelerates initiation, progression and malignant transformation of mammary epithelial tumor cells [17]. In hormone (progesterone)-sensitive endometrial cancer, loss of progesterone receptor expression in the stromal fibroblasts renders resistance to the therapy [18]. Using genetically modified animal model, the progesterone receptor was shown to be epigenetically silenced by tumor cells harboring activated *KRAS* and loss of *PTEN*. While it remains unclear how the tumor cells transform the tumor fibroblasts, these once thought tumor-specific mutations are now found to have critical roles in the fibroblast biology and may therefore affect the response of tumors to drug therapy.

It is possible that tumor cells reprogram the fibroblasts phenotype at the genetic level, especially if these fibroblasts are derived from multi-lineage cells such as mesenchymal stem cells (MSCs). There have been suggestions that tumor fibroblasts may originate from distant sources such as bone marrow-derived MSCs [19]. MSCs may act as a tumor fibroblast precursor cell lineage, as these cells can be mobilized into the tumor by tumor-secreted chemotaxis factors such as chemokine C-C motif ligand 2 (CCL2), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF)[20]. Such niche promotes MSCs transdifferentiation into highly proliferative myofibroblasts that express activation markers α-SMA, fibronectin and tenascin [19, 20]. Through the milieu of secreted factors (including fatty acids), these MSCs-transdifferentiated fibroblasts were shown to induce tumor chemoresistance [21, 22].

Besides MSCs, there are evidence that tumor fibroblasts can be transdifferentiated from tumor and endothelial cells, via epithelial-mesenchymal transition (EMT) and endothelial-mesenchymaltransition (EndMT), respectively [23, 24]. Again, these processes require certain signaling cues from the tumor microenvironment, which involve dynamic activation of multiple growth factor pathways. Transforming growth factor (TGF)-beta, in particular, can almost single-handedly activates pathways implicated in EMT including PI3K/AKT, Smad and RhoA which in turn lead to the loss of epithelial E-cadherin and gain of mesenchymalα-SMA, desmin

and vimentin markers [25]. Yet, the presence of other growth factors such as epidermal growth factor (EGF), hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) within the tumor microenvironment can further collaborate to enhance the transdifferentiation process [26], and this further complicates the effort in delineating the underlying mechanisms. Although observed only in limited models, transdifferentiation of fibroblasts from endothelial cells underscores the plasticity of cells within the tumor microenvironment. Following treatment with TGF-beta and bone morphogenetic protein (BMP), endothelial cells undergo an incomplete mesenchymal transition, expressing mesenchymal markers and retaining endothelial markers such as VE-cadherin, CD31, EGF-like domains 1 (TIE1) and cytokeratins [24]. More intriguingly, these endothelial-derived fibroblasts are predominantly found at the invasive front of the tumors [24], suggesting important roles in tumor aggressiveness.

All these evidence point to the potentially diverse origins of tumor fibroblasts; this may reflect the heterogeneity of fibroblasts expressing different markers, and within different tumor types. Nevertheless, the presence of fibroblast population is difficult to ignore as it constitutes a large proportion of some tumor tissues such as pancreatic adenocarcinoma [27]. Their plasticity and multi-functional properties almost mirror the characteristics of tumor cells, which may serve as the underlying principles for their role in contributing to chemoresistance.

Tumor Fibroblasts Shape the Architecture of Chemoresistance

As in wound healing, fibroblasts in the tumor microenvironment are the primary cells that produce extracellular matrix proteins (e.g. fibronectin and laminin) and matrix regulators (eg. matrix metalloproteinases and matrix protein inhibitors) [9]. These factors are responsible mainly for remodeling the tumor ECM, and hence serve as messengers to relay information between tumor cells and their surroundings. Such communication is dynamic, both in the form of cell-to-cell and cell-to-matrix. Different composition of ECM affects various physical properties such as interstitial fluid pressure and gradients in oxygen and drug concentrations, all of which have been associated with development of chemoresistance [9]. It is well established that tumor cells when grown in 3D matrix show reduced sensitivity to chemotherapy

agents as compared to those that are grown in monolayer culture. This occurs through activation of integrin- and other cell adhesion molecules-mediated survival pathways [9, 28]. In a recent study in lung cancer and squamous cell carcinoma, unbiased genome-wide analysis revealed a unique signature of alteration in gene expression, whereby genes associated with cell adhesion and defense mechanisms, rather than DNA repair, were affected when tumor cells were grown in 3D as compared to 2D culture [29]. Consequently, this yields a significant increase in radio- and chemo-resistance in tumor cells grown in ECM-enriched environment. In some cases, the support provided by the ECM affects mainly the survival of tumor cells, rather than other cellular processes such as cell proliferation and migration. When myeloma cells were cultured on fibronectin, β integrin-mediated amplification of interleukin-6 (IL-6) resulted in an increase of anti-apoptotic Bcl-2 and a decreased of pro-apoptotic Bim protein expression, suggestive of enhanced survival of the tumor cells [30]. Endometrial cancer cells grew very slowly on Matrigel as shown by reduced BrdU incorporation, but their response to progesterone was diminished markedly [31].

The process by which ECM regulates tumor cells response to chemotherapy may be protein-specific. In a leukemia model, β1 integrin induced resistance to doxorubicin through binding to collagen but not fibrin, by upregulating the expression of ATP-binding cassette C1 (ABCC1) protein [32]. Similarly, partnership between β1 integrin and fibronectin results in p27-mediated G1 arrest, and hence development of resistance to etoposide in myeloma cells [33]. It is quite clear that fibroblasts-secreted ECM provides not only biomechanical support, but also information channels for the tumor cells to communicate with their surroundings.

Tumor Fibroblasts-Derived Soluble Factors Confer to Increased Tumor Cell Survival

One of the key activities of fibroblasts is to produce a reservoir of soluble growth factors and cytokines that act in an autocrine or paracrine fashion. These factors are well known for their multiple effects on the epithelial cell survival, including the tumor cells [14]. Although these factors may have overlapping functions, their mechanisms in mediating chemoresistance could be tumor type-specific. In pancreatic cancer, fibroblasts-produced CXCL12

(also known as stroma-derived factor-1, SDF-1) induced transcription of sonic hedgehog (SHH) expression through nuclear factor kappa B (NFκB) activity [34], and hedgehog inhibition by a cyclopamine analog (IPI-296) depleted the stromal tissue and markedly improved delivery of gemcitabine in an animal model [35]. Moreover, abrogation of hedgehog signaling is associated with reduced *stemness* potential of pancreatic tumorspheres and this translated to decreased chemoresistance [36]. Targeting hedgehog signaling in pancreatic cancer may not appear as effective if the tumor tissue is hypoxic, as decreased sensitivity to gemcitabine and 5-fluorouracil was observed when used in combination with hedgehog inhibitors [37]. In cholangiocarcinoma, however, hedgehog signaling was activated by fibroblasts-secreted platelet growth factor BB (PDGF-BB), which rendered reduced cytotoxicity to TRAIL [38]. Hence, the action of soluble factors secreted by tumor fibroblasts may depend greatly on the biology of the tumor cells. It is, therefore, important to determine the exact mechanisms by which these fibroblasts secretion can affect the response of tumor cells to different therapy. As most studies were focusing on selected one or two factors found abundantly in their model, it is worthwhile to note that the synergistic effects between multiple secretory factors may render a different landscape of mechanisms to induce chemoresistance.

Due to many resemblances in function between tumor fibroblasts and activated fibroblasts from benign, inflammatory conditions, it is reasonable to think that benign fibroblasts secretion may exert similar pro-tumorigenic effects including inducing chemoresistance. However, there is accumulating evidence stating otherwise. A classic example was shown in prostate cancer, whereby non-tumorigenic benign prostatic hyperplasia (BPH) cells become tumorigenic after co-inoculated with tumor fibroblasts in a renal capsule implantation, but not when inoculated with benign fibroblasts [39]. Data from our laboratory and others showed that endometrial cancer cells increased their proliferation and resistance to hormonal therapy after exposed to the secretion from tumor fibroblasts, while contrasting effects were seen with the secretion from the benign counterparts [40, 41]. The pro-survival effects were mediated by activation of phosphoinositide 3-kinase (PI3K)/AKT and MAPK signaling, as antagonism using specific inhibitors reversed the tumor fibroblasts-mediated cell proliferation [41]. Such contrasting phenotypes may be explained by the increase levels of pro-inflammatory cytokines (IL-6, IL-8 and macrophage chemoattractant protein (MCP-1)) secreted by these primary fibroblasts established from individual tumor tissues, which were secreted at much lower levels by those from benign tissues [41]. The magnitude of secretory factor levels may be one of the reasons, but temporal effects and

readiness in recipient (tumor) cells to be activated by these factors may also be possible [42]. Hence, it is crucial to first critically characterize the secretome profile in fibroblasts from different pathological conditions. Then, delineation of the signaling pathways orchestrated by these co-existing factors in the tumor microenvironment may potentially provide venues for understanding the development of chemoresistance by tumor fibroblasts. Some of the studies using co-culture studies that demonstrate the role of fibroblast secretion in mediating chemotherapy resistance are summarized in Table 1.

Table 1. Fibroblast-mediated resistance to chemotherapy

Tumor	Cell type	Chemotherapeutic agent	Reference
Pancreas	T3M4, PT45-P1	etoposide	[43]
Melanoma	1205Lu	cisplatin	[30]
Breast	MCF-7	tamoxifen, fulvestrant doxorubicin, PARP inhibitor	[44]
Multiple myeloma	U266, NCI-H929	bortezomib	[22]
Cholangio carcinoma	KMCH-1, KMBC, HuCCT-1, TFK-1, Mz-ChA-1	TRAIL	[38]
Colon	Primary cells	irinotecan, IL-1 beta	[45]
Lung	NCI-H460	VP-16	[46]
Various tumor types	45 cell lines	16 agents	[47]

Tumor Fibroblasts Contribute to EMT Induction

Epithelial-mesenchymal transition (EMT) is an important process during development by which epithelial cells acquire mesenchymal, fibroblast-like properties and show reduced intercellular adhesion and increased motility. Accumulating evidence points to a critical role of EMT in tumor progression, via which tumor cells acquired aggressive properties including resistance to

therapy [48, 49]. EMT induction is mainly mediated by selected differentiation-related transcription factors, including Snail, Twist and zinc finger E-box-binding homeobox (ZEB)[49]. While activation of these transcription factors are thought to be regulated by oncogenic pathways, such as Ras, Src and Wnt [50-52], recent evidence suggests that tumor fibroblasts-secreted growth factors and cytokines may also induce EMT [53-55].

In an *in vitro* direct co-culture model, fibroblasts derived from the interface zone of breast cancer tissues in comparison to those from adjacent benign area, demonstrated greater capacity in inducing an EMT in MCF-7 breast cancer cell line, as indicated by decreased E-cadherin and increased vimentin and N-cadherin expression [56]. In a prostate carcinoma model, tumor fibroblasts induced EMT via a pro-inflammatory signature that is highly dependent on cycloxygenase (COX)-2 activities [53]. Similarly, tumor fibroblasts-induced inflammatory reaction was accounted for the EMT observed in a transgenic pancreatic adenocarcinoma mouse model [54]. In this particular work, EMT and invasiveness were most abundant at inflammatory foci and that leads to increased circulating cancer cells. Although induction of chemoresistance was not the main focus in this work, it is evident that the presence of tumor fibroblasts at close proximity was necessary to induce EMT at both the benign and cancer stages [54]. In fact, tumor fibroblasts exerted greater potency in inducing EMT via direct cells contact, rather than via paracrine signaling molecules, although a reduced proliferation was observed in the tumor cells [57].

Despite extensive studies on EMT in cancer, the role of EMT in tumor progression and chemoresistance *in vivo* remains debatable, as we lack pathological evidence from clinical samples to correlate with the molecular alterations observed in pre-clinical models [58]. More studies are warranted to explore if correlation exists between tumor cells with EMT markers and their response to chemotherapy in cancer patients.

Strategies to Overcome Tumor Fibroblasts–Mediated Chemoresistance

Given the different attributes by which tumor fibroblasts mediate chemoresistance, it is attractive to develop treatment strategies that abrogate the communication between tumor and fibroblast cells. The different chemoresistance-induced mechanisms exploited by the tumor fibroblasts offer

many potential ways to re-structure the network. One obvious strategy is to remove the fibroblasts from the microenvironment. Extensive proliferation of tumor fibroblasts (also known as desmoplasia) can affect delivery of chemotherapy agent into the tumor. Depletion of stromal fibroblasts, via blocking of hedgehog cellular signaling pathway with IPI-926, leads to a transient increase in intratumoral concentration of gemcitabine and hence, a transient stabilized disease in the gemcitabine-resistant KrasLSL.G12D/+;p53R172H/+; Pdx-Cretg/+ (KPC) pancreatic tumor mouse model [35].

However, not all hedgehog inhibitors resulted in an attenuation of tumor stroma, despite remarkably inhibited tumor growth [59]. There are ongoing attempts to translate this promising strategy to the clinic. Despite some encouraging Phase I clinical data, a Phase II trial of gemcitabine plus saridegib, a hedgehog inhibitor, in metastatic pancreatic cancer showed a higher rate of progressive disease and a shorter overall survival in the intervention arm, leading to an early halt [60]. Clinical evidence for nab-paclitaxel in combination with gemcitabine offered a more promising strategy to eliminate desmoplasia in pancreatic tumors, targeting SPARC protein expressed by both the tumor and endothelial cells [61]. Taken together, activated tumor fibroblasts within the pancreatic tissues may be relying on redundant pathways to survive and proliferate, and we may be still far from perfecting our clinical skills to design and interpret potential determinants and predictors of efficacy when targeting the fibroblasts within the tumors.

Following a challenge with cytotoxic agents or small molecule inhibitors, tumor fibroblasts may release various pro-survival growth factors that allow rapid adaptation to overcome the therapy-induced cytotoxicity. For example, treatment of triple negative breast cancer with MEK inhibitor was associated with increased signaling by platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR) and HER2 [62]. Activation of different receptor kinase receptors simultaneously may indicate the crucial role of a common downstream effector that is critical for that particular tumor cells to survive. Designing activators/inhibitors that selectively target this common effector may be proven useful. A recent study has utilized a large scale RNAi screen to identify MED12, a regulator of TGF beta signaling, to be the common mediator of resistance to multiple kinase inhibitors including BRAF, ALK and MEK inhibitors [63]. Loss of MED12 was associated with increased TGF beta signaling, which thought to induce EMT and confer resistance to therapy [63]. Hence, it is reasonably possible to determine potential drug target despite the complexity from activation of redundant signaling pathways. Detailed investigation is therefore necessary to

understand the step-by-step mechanistic events leading to the development of chemoresistance for different therapy in different tumor type.

Recently, nanomedicine or therapeutic nanoparticles have emerged as an innovative and promising alternative to overcome drug resistance [64]. Due to the almost indefinite chemical properties of nanovehicle, it is possible to load various targeted agents into such nanometric delivery system.

Shapira et al. has recently proposed a quadrugnostic platform, whereby an ideal nanomedicine will contain a selective targeting moiety to the tumor tissue, a diagnostic imaging aid to localize the tumor tissue, a cytotoxic small molecule or therapeutic biological, and lastly, a chemosensitizing agent aimed to neutralize any chemoresistance induced by the tumor microenvironment [64].

Such strategy is exploiting the nano-properties of the vehicle to accumulate in the tumor microenvironment, before releasing the therapeutic cargoes that can interfere with the tumor-fibroblast cells communication. A good example for targeting tumor fibroblasts was recently shown using a simpler nanotherapeutic approach. Docetaxel-conjugate nanoparticles, also known as Cellax, are 120 nm particles containing docetaxel and polyethylene glycol (PEG) conjugated to acetylated carboxymethycellulose. In two different orthotopic breast tumor models, treatment with Cellax remarkably depleted up to 80% of alpha-SMA-expressing fibroblasts within the tumor tissues, a significantly greater anti-stroma effect compared to native docetaxel and nab-paclitaxel [65].

Both tumor perfusion and vascular permeability were enhanced significantly, accompanied with a considerable reduction in tumor matrix and tumor interstitial fluid pressure [65]. At least 85% of Cellax administered were delivered to α-SMA-positive fibroblasts, resulting in a rapid depletion of tumor stroma (within 24-hours) and a significant reduction in metastatic lesions [65].

These effects were not seen with docetaxel alone or nab-paclitaxel treatment, strongly suggesting that co-targeting the tumor fibroblasts can impact the drug delivery and hence the efficacy of the cytotoxic agents.

It should be emphasized that tumor fibroblasts are not the only non-tumor component within the tumor microenvironment. Although not covered in this review, overcoming fibroblasts-mediated chemoresistance may require concurrent strategies to target other host-derived factors, for example the macrophages and lymphocytes.

Given the emerging enthusiasm in cancer immunotherapy, it is anticipated that development of fibroblasts- and immune-modulatory agents to complement tumor-targeted therapies will be an exciting area of research.

Conclusion

All anti-cancer drugs used in the clinic are capable of noticeably suppressing tumor growth; yet, each suffers from rapid development of resistance programs. Distinct microenvironment landscaped by tumor fibroblasts can provide sanctuary to the tumors, which contributes substantially to tumor cell survival and eventually relapse and therapy failure. Consequently, tumor cells are no longer the only player responsible for the induction of chemoresistance. Addition of tumor fibroblasts into the paradigm has change the way we look at the failed chemotherapy. Our own body cells, although unwillingly, are partly responsible for the lack of therapeutic efficacy. Strategies to target the bi-directional communication between tumor and fibroblast cells should be emphasized and considered in future cancer drug design and development.

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