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Tumor talk: understanding the conversation between the tumor and its microenvironment

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Abstract

It was once believed that tumor growth, progression, and metastasis were intrinsically driven by the tumor. Instead, recent research has demonstrated that a solid tumor is surrounded by a complex matrix of cells, particularly fibroblasts, which support and even promote tumor progression. This matrix of stromal cells, also known as the tumor microenvironment (TME), plays a critical role in cancer and may represent a novel therapeutic target. As such, understanding the complex nature of how the tumor initiates and maintains communication, or a "conversation", with the TME is the focus of current investigations. We have previously shown that the most prevalent mutation found in melanoma, $BRAF^{V600E}$, results in increased expression and secretion of several growth factors, cytokines, and matrix metalloproteinases, including factors that are able to activate fibroblasts. Targeted inhibition of the $BRAF^{V600E}$ mutation resulted in a decrease of secreted proteins into the TME and suggests that targeting the tumor also modifies the TME. Overall, this work, in combination with several additional studies discussed herein, provides strong evidence for the potential therapeutic benefits of targeting the TME, particularly signaling pathways within the fibroblasts, in conjunction with the tumor. This approach may result in extended drug resistance free survival, reduction in metastasis, and improved cytotoxic drug delivery.

Keywords

activated fibroblasts; tumor microenvironment; $BRAF^{V600E}$; vemurafenib; IL-1β

The tumor microenvironment - more than structural support

It is well established that tumors are incredibly complex tissues comprised of a heterogeneous mixture of cells, and that aberrant gene expression and mutations in critical genes are major contributors to tumor development and progression. However, the picture of what influences tumor growth, and especially tumor metastasis, is a dynamic field, one that has expanded to include the cells that surround and interact with the tumor, i.e., the tumor microenvironment (TME). This microenvironment is a rich and robust mixture comprised of fibroblasts, leukocytes, pericytes, endothelial cells, and extra-cellular matrix[1]. No longer considered a supportive bystander, the TME acts as an active participant in a constant

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conversation with the tumor (Figure 1). A growing body of evidence has revealed that tumor cells do not act alone to promote tumorigenesis, progression, angiogenesis, and metastasis but instead collaborate with an activated TME[2–4].

Under normal conditions fibroblasts, which comprise the largest cellular component of the TME[4], exist in an inactive quiescent state and form a structural network by synthesizing several components of the extracellular matrix (ECM), including collagens, laminin, and fibronectin[4–6]. During wound healing and fibrosis, the fibroblasts become activated, which elicits tissue remodeling and expression of surface markers such as α–smooth muscle actin (α-SMA), Platelet-derived growth factor receptor (PDGFR), fibroblast specific protein (FSP)-1, fibroblast activation protein (FAP), as well as stromal-derived factor-1 (SDF-1: CXCL12) and its receptor CXCR4[1, 6–9]. Upon the completion of wound healing, the majority of the fibroblasts are removed by apoptosis[6, 10]. However, since tumors are often referred to as “wounds that do not heal”, they co-opt fibroblasts such that they become continuously activated and actually promote tumorigenesis[11, 12].

Once activated, these fibroblasts are referred to as cancer-associated fibroblasts (CAFs) and play an integral role in tumor-stromal interaction in several ways[13, 14]. First, fibroblasts contribute to tumor cell growth and invasion by increasing production of ECM proteins and proteases, thereby promoting tumor growth by degrading and remodeling components of the ECM[4, 5]. Next, fibroblasts suppress the immune response by recruiting inflammatory cells (such as monocytes and macrophages) to the tumor as well as by modifying immune cell function[4, 15]. Lastly, fibroblasts release growth factors and cytokines, including vascular endothelial growth factor (VEGF), transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), platelet derived growth factor (PDGF), SDF-1, cyclooxygenase 2 (COX-2), and several interleukins (IL-1β, IL-6, IL-8), which in turn can promote tumor angiogenesis and metastasis[16–18].

An active conversation between the tumor cells and the tumor microenvironment

Accumulating evidence emphasizes the importance of the TME in enhancing the aggressive behavior of several types of cancer, especially melanoma, pancreatic, and breast cancer[17, 19, 20]. The exact players and mechanisms that enable a tumor to activate the TME are not completely understood. In our recent study, we asked what effect the most common mutation found in melanoma, $BRAF^{V600E}$, has on cytokine production by the tumor and how this impacted human dermal fibroblasts *in vitro*[17]. We found that melanoma cells engineered to express $BRAF^{V600E}$ expressed higher levels of the secreted proteins, IL-1β, IL-6, IL-8, and matrix metalloproteinase-1 (MMP-1), than wild-type cellular counterparts. Notably, conditioned media collected from the $BRAF^{V600E}$ melanoma cells promoted stromal fibroblast activation as indicated by an increased expression of SDF-1 (CXCL12) and its receptor CXCR4. Of particular interest, when the $BRAF^{V600E}$ specific inhibitor, vemurafenib, was added to the tumor cells, it reduced the expression of MMP-1 and cytokines IL-1β, IL-6, IL-8, thereby mitigating the activation of the fibroblasts (Figure1). Moreover, we found that exogenous addition of IL-1β was able to rescue expression of these

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cytokines, suggesting that IL-1β could act as a "master regulator" of gene expression in both tumor and stromal cells[17].

It has been demonstrated that IL-1β can induce the expression of several growth factors and chemokines, including SDF-1 and CXCR4[21–24]. Overexpression of SDF-1 and CXCR4 are not only important markers of activated fibroblasts, but also prognostic markers in various types of cancer[25, 26]. The CXCR4/SDF-1 signaling axis is involved in several critical aspects of inflammation and tumorigenesis, including: promoting tumor cell migration, invasion and site-specific metastasis[27, 28]. Notably, CXCR4/SDF-1 signaling has been shown to be pivotal in the trafficking of both normal and cancer stem cells to organs that express high levels of SDF-1, such as the lymph nodes, lungs, liver, and bone[25, 28, 29]. Additionally, several cancers with abundant CXCR4 expression (e.g. breast, ovarian, and prostate cancers, as well as neuroblastoma) have been shown to exhibit increased metastasis in a SDF-1 dependent manner[30]. Tumor progression is also enhanced via CXCR4/SDF-1 signaling. Specifically, CXCR4/SDF-1 activates several critical signal transduction pathways, including: adhesion (e.g. Fak, Paxillin, p130 CAS, PI3K, pMAPK p42/44), transcription factors, and phosphatases[29, 31]. CXCR4/SDF-1 signaling promotes matrix remodeling and invasion via the production of matrix metalloproteinases, especially MMP-1[26, 29, 32]. Considering the large number of studies that demonstrate the critical importance of CXCR4 and SDF-1 in cancer metastasis[28, 31], this interaction has emerged as a highly relevant target for hindering metastatic progression[28]. In fact, several small molecule CXCR4 antagonists, under various stages of development, have shown promising results in reducing CXCR4/SDF-1 signaling, inflammation, and metastasis[28, 33].

It is possible that IL-1β plays an integral role in tumor cells and CAFs as well as in additional stromal components such as macrophages and monocytes. It has been shown that tumor associated macrophages (TAMs), found in renal cell carcinoma (RCC), produced elevated levels of IL-1β and that the resulting high serum levels correlated with advanced disease[34]. Additionally, IL-1β contributed to disease progression by enhancing tumor cell invasion through an induction of expression in MMP-1, MMP-3, MMP-10 and MMP-14[34]. In a separate study, IL-1β, along with several additional proinflammatory cytokine, chemokines, and "protumor" genes, was upregulated in RCC and this elevated expression, which was detected in the plasma of RCC patients and in tumors, was significantly correlated with advanced tumor stages[35]. This study went on to show that in xenograft models, an antagonist of IL-1β and its receptor IL-1R1, not only impaired the expression of protumor genes and functions in TAMs but also reduced tumor growth, angiogenesis, and invasion[35]. Combined, these studies suggest that the tumorigenic effect of the IL-1β/IL-1R pathway is either intrinsic to transformed stromal cells or is mediated by the cross-communication between the tumor cells and stromal cells (e.g., immune cells, endothelial cells, and activated fibroblasts). Moreover, despite limited knowledge of the role that macrophages and monocytes play in promotion of human cancer, these studies suggest that the proinflammatory milieu expressed by TAMs, monocytes, and fibroblasts in the TME directly impacts the gene expression profile of the tumor cells[35]. Additional studies investigating the impact of targeting this subset of the TME through inhibition of IL-1β and its receptor may provide a novel avenue of combined treatment.

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Targeting the stroma – potential approaches to reducing metastasis and extending response to treatment

Identifying the primary molecular signals, or key points of “conversation”, between the TME and the tumor could reveal viable novel approaches to inhibiting tumor growth and metastasis. It is known that tumor cells secrete growth factors and cytokines which recruit, or activate, the stroma and that this produces an autocrine effect where the stroma, in turn, modifies and even enables the proliferative and invasive behavior of the tumor cells[36, 37]. However, despite the advent of multiple therapeutic strategies aimed at eliminating tumor progression, many questions remain as to how targeting the tumor cells impacts the TME. For example, as our study suggests, does treatment with vemurafenib in metastatic melanoma ameliorate the TME such that the TME no longer promotes tumor progression? If so, is it possible that a reduced expression (and secretion) of inflammatory cytokines and growth factors could result in an enhanced or extended normalization or treatment window? This extended treatment window might provide an optimal time for introducing a novel inhibitor. This inhibitor could either A) specifically target activated stromal cells or B) enable a two-pronged approach where the inhibitor is aimed at two different cell populations: tumor cells and fibroblasts.

It is likely that targeting stromal cells, in conjunction with inhibition of the tumor, will be more successful at reducing tumor growth and metastasis than targeting the tumor alone, especially considering that these cells exhibit high genomic stability and thus are less likely to develop drug resistance[38]. Smalley *et al*. proposed three areas on which to focus the stromal therapeutic approach: inhibiting the ECM remodeling capability, blocking adhesive interactions between the stroma and the tumor, and impeding the downstream signaling pathways (especially receptor tyrosine kinase/growth factors)[38]. Although solid tumors are infiltrated by fibroblasts as well as inflammatory and endothelial cells, most of the dynamic interaction between the tumor and the stroma occurs exactly where the majority of the tumors active growth occurs - at the tumor periphery[39, 40]. This is of particular relevance as recent work has shown that CXCR4 expression is significantly increased at the tumor front versus the tumor center[41]. Combined, these data suggest that targeting the CXCR4/SDF-1 signaling axis, at the interface between the tumor and the stroma, could have a considerable impact on destabilizing the tumor-stroma “conversation”, resulting in inhibition of tumor progression and metastasis.

Several therapeutic agents targeting CXCR4, PDGF receptors, IL-1β, IL-6, IL-8, MMP inhibitors, CAF derived growth factors, and CAF derived glycoproteins (such as Tenascin-C) are already at various stages of investigation[1, 33, 42–50]. Optimally, at least one of these agents could prove highly efficacious, especially when used in combination with current treatments. It is also plausible that a novel TME inhibitor could generate a synergistic effect such that it amplifies the therapeutic effect of current drugs, potentially with few side effects. Previous studies have shown that the stroma plays a critical role in determining anticancer drug activity as well as enabling and supporting chemo-resistance[51–54]. In fact, stromal mediated alterations in malignant tumor cells not only correlate with clinical drug resistance but a high-through-put screen of over 3,000 anticancer compounds revealed that

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more than half of these compounds were less active in the presence of the stroma[52]. Thus, it is highly possible that targeting the stroma in conjunction with the tumor could result in a synergistic response of the anti-cancer drug as well as a reduction in clinical drug resistance.

Considering the large heterogeneity of the tumor-stroma interaction, it is unlikely that one single target will be able to stop tumorigenesis and metastasis. However, attaining a better understanding of the "conversation" between CAFs and the tumor, especially elucidating changes in gene expression in CAFs upon activation, may provide critical insights to make anti-cancer therapy more effective as well as to identify novel anti-cancer therapies. There are several key areas of the "conversation" to target: 1.) inhibiting tumor signals being sent to the fibroblasts 2.) inhibiting the return signal from the CAFs 3.) eliminating the CAFs themselves[55].

Arguably, the most beneficial target may prove to be the CAFs themselves. Gonda *et al.* summarized several lines of evidence for targeting CAFs that extends beyond the fact that they can support tumor proliferation, angiogenesis, and invasion[45]. First, CAFs are less likely (than tumor cells) to acquire new genetic mutations, thus they may be less prone to escape or to develop drug resistance due to genomic stability[45]. Secondly, current cancer treatments often lead to residual fibrosis, which suggests adjuvant therapy may be needed to target this fibrosis[45, 56]. Third, CAF derived factors can interfere with anti-cancer therapies, contribute to recruitment of bone-marrow derived cells to tumors, and may prevent effective immune surveillance of anti-tumor response[20, 45, 57, 58]. Lastly, a negative correlation may exist between the level of involvement and activation of the stroma and survival in certain cancers[45, 59].

Although targeting the CAFs directly may prove to be the most efficacious approach, it will likely involve several technical challenges, similar to those encountered when developing tumor cell specific antibodies. However, preliminary studies in pancreatic cancer, a cancer known for its large stromal reaction, have revealed that reduction of stromal cell proliferation can increase distribution of therapeutic agents to tumor cells[45, 60]. Specifically, in a xenograft model of pancreatic cancer, Olive *et al*. showed that when they inhibited stromal proliferation by targeting the hedgehog receptor, they normalized the tumor vasculature enabling enhanced delivery of the therapeutic drug to the tumor[60]. Importantly, these findings correlated with an increase in survival[60]. It may also be possible to inhibit CAF function and proliferation by targeting epigenetic alterations such as DNA methylation[45]. Experiments in mouse models of stroma rich human cancers with demethylating drugs are currently under investigation[61, 62].

In conclusion, understanding the TME and its interaction with the tumor is a complex and dynamic field. In order to significantly reduce the tumor promoting effects of the TME, it may be necessary to reduce the number of CAFs by targeting the tumor signal sent to the stroma, target the CAF signaling back to the tumor, or eliminate the CAFs themselves in order to abolish the "conversation" and help to normalize the TME. One promising area currently under investigation is aimed at understanding and comparing stromal differences across cancer types in order to discern the impact of these differences on tumor progression and cancer prognosis. It is possible that specific cancer types, especially cancers with a

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higher level of stromal interaction, will require an individualized approach to simultaneously target the tumor and the TME. Moreover, although fibroblasts are the predominant cell type surrounding the tumor[4]; the TME is a rich and diverse environment, consisting of a multitude of cells including: endothelial cells, pericytes, leukocytes, extra-cellular matrix. Thus, targeting other stromal components, either separately or in combination with activated fibroblasts is a promising avenue for future investigations, which may lead to significant progress in improving response to treatment for a range of cancer types.

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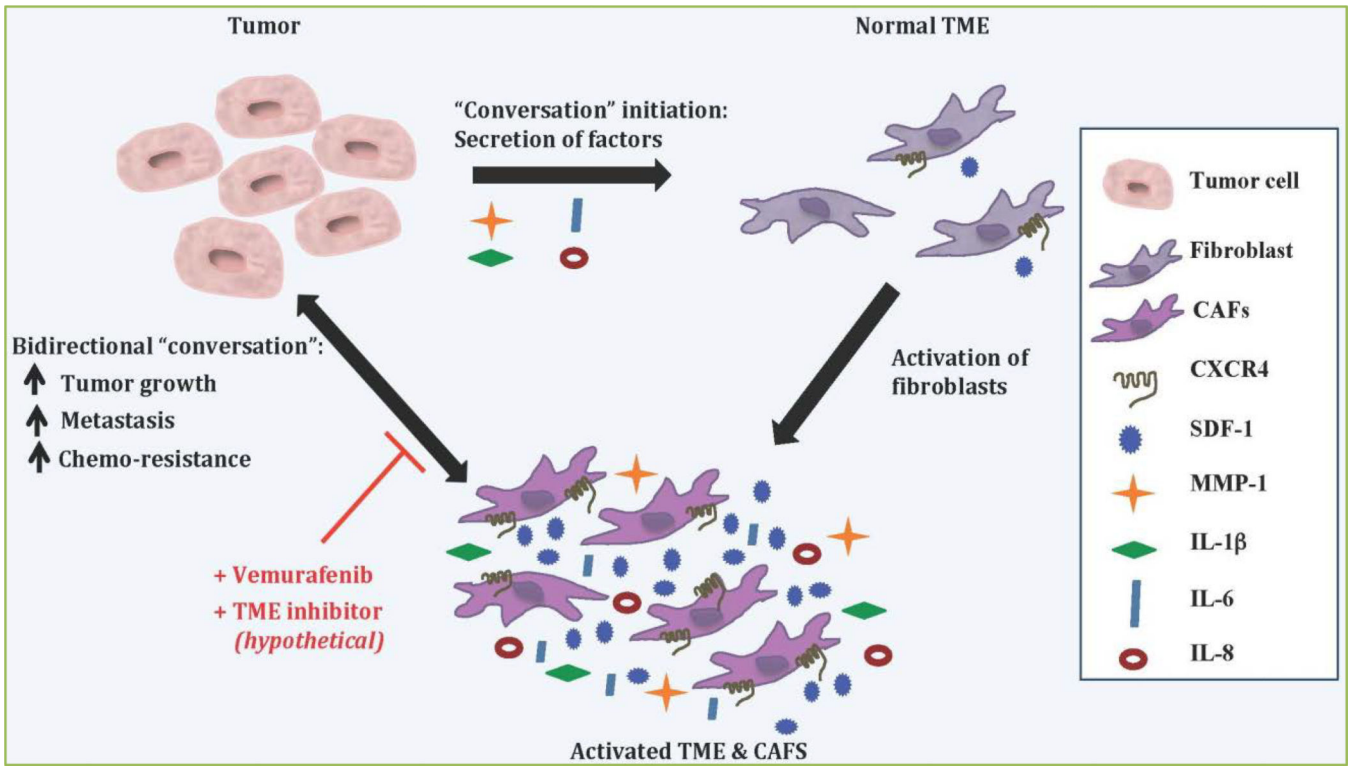

Figure 1. The conversation between the tumor and the stroma

The tumor secretes factors, such as MMP-1, IL-1β, IL-6, and IL-8 into the surrounding tumor microenvironment (TME). These factors activate the fibroblasts that comprise the TME resulting in an increased expression of CXCR4, SDF-1, MMP-1, IL-1β, IL-6, and IL-8. The activated TME, and specifically the cancer-associated fibroblasts (CAFs), engages in a bidirectional "conversation" where it encourages and enables tumor growth, metastasis, and chemo-resistance. In turn, the tumor responds by continuing to secrete activation factors into the TME. Stopping this "conversation" by targeting the tumor cells with an anti-cancer drug such as Vemurafenib while simultaneously utilizing a (hypothetical) TME inhibitor, potentially one that specifically targets the CAFs, could result in an amplified therapeutic response.