doi:10.4149/neo_2013_001

# Tumor microenvironment and the role of mesenchymal stromal cells

*Minireview*

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**Received April 24, 2012 / Accepted May 28, 2012**

Solid tumors are generally composed of two major components: heterogeneous malignant cells and non-malignant stromal part. The latter comprises several types of non-malignant cells of mesenchymal, endothelial and immune origin and together with the extracellular matrix significantly affects the biological properties of the tumor. This minireview is focused on recent advances in the understanding the role of tumor stromal component and its particular cell types in the tumor behavior. It summarizes the impact of mesenchymal stromal cells and the ways of their potential contribution to the tumor biology. As their role in the tumor development and the effects on the tumor cells remain controversial, we review the recent experimental evidence regarding the crucial molecular factors which determine their role in the tumors.

*Key words: tumor microenvironment, human mesenchymal stromal cells, paracrine interaction, malignant cells*

## Tumor microenvironment

Tumor tissue is composed of heterogeneous cell populations that interact within complex signalling networks. It includes extracellular matrix (ECM) and various non-transformed cells (e.g. myoepithelial and epithelial cells, fibroblasts, myofibroblasts and leukocytes). There is a bi-directional interplay between tumor cells and the heterogeneous population of cells surrounding them. Some of these cells exist in the tissue before the cancer development; others are specifically recruited to the microenvironment by the tumor itself. Tumor cells are able to communicate with, and receive signals from various components of the microenvironment that ultimately affect the development and progression of the tumor itself [1; 2].

Many studies have described prominent effect of tumor microenvironment on the behaviour of cancer cells [3]. There occurs a dynamic and reciprocal communication between epithelial and stromal compartments during breast cancer progression. These complicated networks of various cell types promote tumor progression by secreting growth factors, chemokines, and promigratory extracellular matrix components. Originally described by Paget et al. 1889, the concept of "seed-and-soil" has been adopted to describe the importance of the microenvironment for the tumor/metastasis development [4; 5]. Tumors as heterogeneous cellular entities which growth is dependent upon reciprocal interactions between genetically altered initiating cells ("seed") and the dynamic stromal microenvironment ("soil") in which they reside [6]. How the microenvironment differs between normal and cancer tissues attracts growing research attention. The importance of several components of the "soil" in regulating tumor growth has been emphasised: the extracellular matrix; stromal cells and their growth factors and inhibitors; microvessels and angiogenic factors and inflammatory cells [7]. Understanding the crosstalk between tumors and their microenvironment and also the network of cytokines and secreted growth factors may lead to important new therapeutic approaches in controlling the growth and metastasis of tumors.

## Cellular components of tumor microenvironment

**Fibroblasts.** Fibroblasts themselves are non-vascular, non-epithelial and non-inflammatory cells. They form the basic cellular component of connective tissue and they are found

in various proportions in different types of carcinomas. Fibroblasts play a key role in the deposition of the extracellular matrix (ECM) and have generally a low rate of proliferation under physiological conditions in normal tissues and organs. They exhibit a highly proliferative nature during wound healing, secrete large amounts of ECM components and acquire a contractile, myofibroblastic phenotype. Fibroblasts within the tumor stroma have been referred to as cancer associated fibroblasts (CAFs) [8; 9]. This term comprises at least two distinct cell types. First type represents cells with similarities to the fibroblasts supporting most normal epithelial tissues and the second ones are myofibroblasts that are markedly different from tissue-derived fibroblasts. In the activated state, CAFs produce a variety of cytokines, growth factors and ECM proteins by which they alter both the tumor cells and the stromal microenvironment and promote tumor progression. They produce also ECM proteins and thus determine the biophysical properties of the ECM (thereby indirectly influencing cancer cell motility and invasion modes) [10]. CAFs may also act as a barrier against tumor-infiltrating immune cells and the access of anti-cancer drugs to the tumor cells. They facilitate cell contacts and motility, and stimulate secreted proteins which in turn stimulate invasiveness, angiogenesis and tissue remodelling [11]. CAFs may also originate from mesenchymal stromal cells as these were demonstrated to posses the capability to differentiate into the CAFs [12; 13].

Fibroblasts within the stroma of breast carcinomas can be recruited from different sources, and one of them might be circulating or tissue-resident MSCs (mesenchymal stromal cells). Fibroblasts within invasive breast carcinomas contribute to tumor promotion via secretion of SDF-1α and HGF. They promote tumor growth and neoangiogenesis through a SDF-1/CXCR4-dependent recruitment of endothelial progenitor cells (EPC). There are several secreted factors, such as VEGF, SDF-1α, soluble c-Kit ligand (SCF), and matrix metalloproteinase 9 (MMP9) that have been implicated as possible regulators of EPC proliferation, mobilization and migration [2; 11]. Endothelial progenitor cells (EPCs) differentiate into mature circulating endothelial cells via signalling through receptor for vascular endothelial growth factor-2 (VEGFR2). They contribute to tumor vasculature, but the extent appears to be highly dependent on the experimental model [14].

**Tumor-associated adipocytes.** The most abundant cells in the breast tumor stroma are mature adipocytes. They originate from MSCs and were mainly considered as an energy storage depot. Nowadays there is strong evidence showing that mature adipocytes are able to enhance invasive phenotype of tumor cells. Coculture of breast tumor cells with mature adipocytes shows that cocultivated adipocytes generally exhibit overexpression of inflammatory cytokines (IL6, IL1β) and proteases [15].

**Immune inflammatory cells.** Infiltrating cells of the immune system can be found in most if not all neoplastic lesions [16]. They operate in conflicting ways: both tumor-antagonizing and tumor-promoting. The list of tumor-promoting cells includes macrophage subtypes, mast cells, neutrophils, as well as T and B lymphocytes. Inflammatory cells produce a lot of proangiogenic signalling molecules like tumor growth factor EGF, vascular endothelial growth factor VEGF, chemokines and cytokines that amplify the inflammatory state. Macrophage infiltration is a common feature of solid tumors and it can promote tumor survival and neovascularisation. Moreover, high density of tumor-associated macrophages (TAMs) correlates with poor prognosis in cancer patients. TAMs are recruited into tumors as monocytes by chemotactic cytokines and growth factors from the bloodstream and they subsequently differentiate into tissue macrophages [17; 18]. It is well accepted that TAMs are required for tumor cell migration, invasion, and metastasis formation. Tumor cells exposed to TAMs' prometastatic activity exhibit increased invasiveness and an enhanced capacity to adhere to endothelial cells (ECs) and thus eventually (and indirectly) facilitate trans-endothelial migration. TAMs generally accumulate in the hypoxic areas of the tumor which in turn triggers a pro-angiogenic program in these cells. Thereby, TAMs promote the angiogenic switch and neovascularization as well as malignant transition of the tumor cells by secretion of specific proangiogenic factors (VEGF, IL-1b, TNF-a, angiogenin, semaphorin 4D), or indirectly through the release of MMP-9 [19].

Some reports pointed to antitumor activity of inflammatory cells. No increased risk of cancer is associated with some chronic inflammatory conditions [20]. Appropriately activated macrophages are able to kill the tumor cells and elicit cancer-destructive inflammatory responses. The balance of protumor and antitumor macrophage activity has been attributed to the NF-κB signalling regulation [21]. The activation of innate immunity also plays role in triggering "good" antitumor effects although precise mechanism how to change tumor-promoting ($T_H2$ and M2 macrophage) microenvironment to tumor inhibiting ($T_H1$ and M1 macrophage) remains to be understood [22].

**Endothelial cells.** Endothelial cells represent another stromal constituent forming the tumor-associated vasculature [17]. Cell-biological program of "angiogenic switch" is responsible for activation of quiescent endothelial cells and new blood vessel formation. It is also functionally implicated in tumor-associated angiogenesis [23]. Research on gene expression profiles of the tumor-associated endothelial cells is likely to reveal the key pathways leading to conversion from normal endothelial cells to tumor-associated endothelial cells. Endothelial progenitor cells can be recruited to tumors from the circulation, incorporate into tumor vasculature and differentiate into mature endothelial cells [24]. Some authors suggested that the process of trans-differentiation can contribute to this process [14]. MSC-derived fibroblasts are thought to have the capability to (trans)-differentiate into endothelial-like cells and pericyte-like cells, which would stimulate tumor growth by the formation and stabilization of tumor blood vessels. Indeed, MSCs have the capability of tube formation ([25] and our unpublished data) thus indi-

cating that they seem to be prone to (trans)-differentiation into endothelial-like cells. In spite of that, MSCs secrete abundant amounts of the pro-angiogenic factors that may contribute to the development of tumor vasculature, its stabilization and development of mature endothelial cells [26; 27].

**Mesenchymal stromal cells (MSCs).** MSCs are a subset of non-hematopoietic cells that display homing and engraftment potential to the injured or damaged tissue in a number of pathological conditions such as inflammation, tissue repair and also neoplasia [28; 29]. There is not a single cell surface marker that uniquely characterizes MSCs. They are typically negative for the hematopoietic cell markers CD34, CD45 and positive for the surface markers CD44, CD73, CD90, CD105, CD106, and STRO-1 [30; 31].

MSCs can be recruited to tumors from various normal tissues because tumor cells secrete growth factors and cytokines that promote MSC homing in primary tumors. Mesenchymal and progenitor cells were found to transit into tumor from bone marrow [32]. Adipose tissue is also a rich source of these cells and adipose tissue-derived MSCs can contribute to tumor stroma [33]. The adipose tissue-derived MSCs share a number of important characteristics with bone marrow-derived MSCs, including cell surface marker expression, plastic adherence, and the capacity to differentiate into cells of mesenchymal lineage (i.e. fat, bone, muscle, and cartilage) under the appropriate conditions [34]. They have also been reported to influence the morphology and proliferation of cells within their vicinity through both cell-to-cell interactions and the secretion of chemoattractant cytokines and paracrine factors [28]. The role of MSCs in tumor growth is quite diversified and includes a regulatory function on tumor cell proliferation, metastasis, angiogenesis and migration. MSCs have four main effects on tumor cells:

- MSCs have an immunomodulatory role which can indirectly affect tumor growth. MSCs display suppressive effects on both innate and humoral immunity by inhibiting $T_H1$ lymphocytes, dendritic cells, B cells, and NK cells [35]. Djouad *et al.* have reported immunologic mechanism of MSCs mediated melanoma growth in allogeneic recipients [36].
- MSCs affect cell survival having both supportive and inhibitory effect on tumor growth (reviewed in [37; 38]).
- MSCs contribute to the tumor vasculature by producing angiogenic factors. MSCs secrete many proangiogenic factors, including VEGF, angiopoetin, IL-6, IL-8, TGF-β (transforming growth factor-β), PDGF (platelet derived growth factor), bFGF (basic fibroblast growth factor), and FGF-7 (fibroblast growth factor type 7). All these cytokines are able to alter the normal tissue homeostasis stimulating the formation of new blood vessels as well as activating and recruiting stromal fibroblasts (reviewed in [39]).
- MSCs can promote tumor cell motility, cancer progression and metastasis to distant organs via the production of CCL5 (chemokine, C-C motif ligand 5, RANTES-regulated upon activation, normal T-cell expressed and secreted) [40; 41]).

*MSCs effect on tumors.* MSCs and their effect on tumors was studied in detail and ambivalent MSC action on tumors was described: both tumor-promoting and tumor-inhibitory (comprehensively summarized recently in [38; 42; 43]). The effect of MSCs on tumor cells depends on their origin, their degree of differentiation, dose, administration route and the type of cells they interact with [38; 42]. There are many mechanisms responsible for these observations, such as mutual autocrine and paracrine signalling via secreted chemokines, modulation of apoptosis and vascular support.

*MSCs-mediated tumor promotion***.** Mechanisms responsible for MSCs mediated tumor growth support cover both direct impact on tumor cell proliferation [44] and vasculogenesis [45; 46]. Moreover, the coinjection of mesenchymal cells was reported to increase the tumor incidence and size via SDF-1/CXCR4 signalling [47; 48]. In our experiments we have been using human adipose tissue-derived MSCs in the context of cancer gene therapy [49]. These studies were performed with same type of AT-MSCs in immunodeficient mouse models *in vivo.* Thus they eliminate at least some variables and inadvertently enable us to evaluate the role of AT-MSCs in tumor development. Initially we have reported that AT-MSCs had an indifferent role in development of tumor xenograft from human colorectal carcinoma derived cells HT-29 *in vivo* [50]. We were able to detect less then 4 % of engrafted transgenic AT-MSCs in subcutaneously grown xenotransplants which seems to be below the proportion capable of substantially affecting tumor volume [38]. So far we have reported AT-MSCs mediated tumor support for the three different tumor cell types *in vivo*. Human AT-MSCs significantly shortened the time to xenotransplant onset in human prostate carcinoma cells PC3 and melanoma cells M4Beu and A375 [48; 51]. The average xenotransplant volume was significantly increased in A375 melanoma xenografts when coinjected with higher then 10% proportion of AT-MSCs. Moreover, we have shown that AT-MSCs could abrogate tumor dormancy of human melanoma A375 xenotransplanted cell upon systemic administration [48]. Another studies have reported decreased apoptosis as another effect of MSCs on tumor cells that might contribute to growth stimulation *in vivo* [52]. There is growing evidence that MSCs can affect proliferation and suppress apoptosis of breast cancer cells [27; 48; 53]. Greco *et al.* recently suggested the role of IL-1 produced from MSCs to affect chemosensitivity in SKBR3 cells [54]. Wide range of biological effects mediated by MSCs on tumor cells can be attributed to their specific molecular profile and a unique pattern of the complex signalling networks in response to soluble metabolites released in the their microenvironment [27].

*MSCs-mediated tumor inhibition.* Several reports mentioned that human MSCs mediated tumor growth inhibition, increased latency, decreased tumor size and/or metastasis [38]. Multiple mechanisms were suggested to mediate this interaction such as AKT (serine/threonine protein kinase) signaling

[55], WNT signaling [56], DKK-1 (Dickkopf-1 protein) secretion [57] or G1 cell cycle arrest [58]. We reported two different types of tumor cells – human glioblastoma derived 8-MG-BA and human medullary thyroid carcinoma derived TT – which were inhibited in proliferation by AT-MSCs *in vitro* and AT-MSCs inhibited the growth of tumor xenotransplants *in vivo* [48; 59].

In summary these findings indicate that tumor cells are important determinants of the mutual tumor-stromal cell interplay within the complex heterogeneous tumor microenvironment.

**Interactions of tumor cells with their microenvironment**

Tumor cells are subject to mechanical and nutritional stress by high pressure and oxygen deprivation so they participate in the creation of a favourable microenvironment by interacting with stromal cells and triggering the homing of a variety of cells to the tumor site. Tumor microenvironment is essential for tumor cell proliferation, angiogenesis, invasion and metastasis through its provision of survival signals, secretion of growth and pro-angiogenic factors [1; 17]. Nevertheless, there are studies that pursue the idea of a leading role for tumor stromal environment in the initiation of carcinomas, not being just a supporting bystander [8]. Interactions of genetically altered tumor cells with the extracellular matrix (ECM) of the tumor microenvironment and reactive non-neoplastic cells control many aspects of tumorigenesis such as epithelial-mesenchymal-transition (EMT), migration, invasion, metastasis formation, neovascularisation, apoptosis and chemotherapeutic drug resistance [60].

**Epithelial-to-mesenchymal transition (EMT)**

EMT is a multistep process by which transformed epithelial cells can acquire the abilities to invade, resist apoptosis, and disseminate through the acquisition of stem like properties, including loss of cellular polarity, adhesion and proliferation. The EMT program regulates a particular type of invasiveness that has been termed "mesenchymal". It is associated with epithelial cell depolarization and adoption of mesenchymal phenotype characterized by an enhanced migratory ability, reorganization of cytoskeleton to acquire more spindle-shape morphology and increased expression of extracellular matrix protein. Cancer cells may enter into an EMT program only partially, thereby acquiring new mesenchymal traits while continuing to express residual epithelial traits. The EMT is now thought to play a fundamental role in tumor progression and metastasis formation, where individual cells detach from primary tumors and migrate following extracellular matrix network. Current research analyzes the contributions of cancer-associated fibroblasts and bone-marrow derived mesenchymal stem cells to EMT in tumor cells [17; 61]. MSCs are capable to integrate into breast cancer mammospheres, shift their phenotype to more mesenchymal-like and decrease E-cadherin surface expression [62]. MSCs were also shown to stimulate EMT in human breast cancer cell lines MDA-MB-231, T4-7D and SKBR3 *in vitro* [63].

The EMT can be induced during cell culture by extracellular matrix components and growth factors, such as transforming growth factor-β (TGF-β), hepatocyte growth factor, fibroblast growth factors and many others *in vitro* [64; 65; 66]. EMT is mediated by overexpression of members of the Snail family (SNAI1, SNAI2) and basic helix loop helix family (TWIST) which mediate loss of E-cadherin and gain of N-cadherin expression. Saxena *et al.* demonstrated that the ABC transporters, responsible for efflux of chemotherapeutic drugs, carry binding sites for EMT-inducing transcription factors and overexpression of Snai1 and Twist increases their promoter activity thus linking the EMT process and drug resistance [67].

The EMT process is one of the early steps in the invasion-metastasis cascade through which polarized epithelial cells undergo phenotypic changes and acquire more mesenchymal morphology. Twist plays a crucial role by down-regulating E-cadherin and β-catenin (correlated with EMT), mediate cell motility and invasiveness, promote angiogenesis and correlate with chromosomal instability in breast cancer (reviewed [68]). Snai1 and Snai2 were suggested to be the inducers of EMT, because their expression is associated with E-cadherin repression, process of metastasis and tumor recurrence [69]. Casas *et al.* showed that the induction of Snai2 is required for ability of Twist to promote EMT and interestingly that the entire EMT morphogenesis is blocked in the absence of Snai2 [70].

**Angiogenesis**

Like normal tissue, tumors require sustained input of nutrients and oxygen as well as the ability to exclude metabolic waste and carbon dioxide. Initially, solid tumors are avascular i.e., they do not have their own blood supply, and rely on diffusion from the surrounding vasculature to supply oxygen and nutrients and remove waste products. As the tumor grows, nutrient demand increases until the influx of nutrients through the surface of the tumor is too small to supply the entire mass of cells. A necrotic core of dead cells may develop at the center and eventually the tumor stops growing and reaches a steady state size of 1-3 mm, in which the number of dying cells counterbalances the number of proliferating cells. Growth can resume only if the tumor becomes vascularized i.e., if it becomes permeated with a network of capillaries. An early response of tumor cells to hypoxia (oxygen deprivation) is the expression of genes that induce nearby vessels to grow new capillaries to vascularize the tumor through a process called angiogenesis. These growth factors diffuse from tumor cells to the nearby primary vessels, and initiate activation of endothelial cells that line the blood vessel walls, inducing them to proliferate and migrate chemotactically toward the tumor [7; 71]. The angiogenic switch is triggered by expression of angiogenic proteins including VEGF, fibroblast growth factor

(FGF), platelet derived growth factor (PDGF), endothelial growth factor (EGF), by metabolic or mechanical stress, genetic mutations or by hypoxia. There is a repertoire of cell types which play a crucial role in pathological angiogenesis. These include the cells of innate immune system – macrophages, neutrophils, mast cells and myeloid progenitors. This results in the formation of a new capillary network that extends from the primary vessel into the growth factor-secreting tumor, thereby bringing essential nutrients to the tumor and providing a shorter route for the spread of tumor cells to other parts of the body [72].

**Metastasis**

Cellular components of tumor microenvironment contribute also to tumor metastasis [73]. Matrix invasion is a crucial prerequisite for metastasis and is regarded to be largely a mechanical process dependent on the expression of adhesion molecules and matrix degrading enzymes [19]. Brabek *et al.* showed that the migration strategy and efficiency of cancer cell invasion is determined the architecture and composition of the microenvironment in terms of structural and biochemical properties of the ECM (fiber network morphology collagen content, fiber thickness, extent of intrafibrillar crosslinks, and the ratio mesh size-diameter of the migrating cell) [74]. Tumor cells are capable of mechanosensing the composition of the ECM which is facilitated by integrin-mediated adhesions and downstream mechanosensor proteins such as focal adhesion kinase. The mechanical properties of the ECM can be remodeled by tumor cells leading to characteristic stiffening of the tumor tissue through collagen crosslinking and increased focal adhesion formation in breast cancer. Inhibition of integrin signalling represses invasion and hence integrins and their downstream mediators represent viable therapeutic targets for anticancer treatment [75]. Breast tumors constantly interact with different compartments of their microenvironment and tumor behaviour is largely dictated by tumor stroma [3; 33]. MSCs, an important component of the tumor microenvironment, can interact with breast cancer cells via direct contact or paracrine regulatory mechanism. Karnoub *et al.* have shown by comparing the effect of MSCs on xenotransplant growth that the tumor proliferation was not generally increased *in vivo*. However, MSCs mixed with breast cancer cells before subcutaneous injection could favour neoplastic cell dissemination by secreting the chemokine CCL5 [40]. They suggested that stromal cells may enhance metastasis dissemination in breast cancer.

**Migration and invasion**

The migration of single cell is the best-studied mechanism of cell movement *in vitro* and it is known to contribute to many physiological motility processes *in vivo*, such as development, immune surveillance and tumor metastasis. The term invasion we use to describe all forms of cell migration through three-dimensional tissue that involve a change in tissue structure and, eventually, tissue destruction. Tumor cells utilize different strategies for migration, namely collective versus individual movement. During collective movements the tumor cells retain their intracellular junctions while individual migration strategies can be performed either mesenchymal-like or amoeboid. Both strategies are interchangeable with bidirectional transition and differentially controlled by growth factors. The tumor stroma plays a central role in tumor migration and invasion. Several cell types present within the tumor microenvironment including tumor associated macrophages and cancer associated fibroblasts appear to have important roles in tumor invasion into surrounding tissue [73; 76]. Rhodes *et al.* have shown significant increase in proliferation and migration of breast cancer cells MCF-7 in response to soluble factors secreted by MSCs [77].

**Cytokine component of the tumor microenvironment**

Signals from the microenvironment have a profound influence on the maintenance and/or progression of epithelial cancers. The assembly and collective contribution of the certain cell types constituting the tumor microenvironment are orchestrated and maintained by reciprocal heterotypic signalling interaction [17]. Human chemokine system currently includes more than 40 chemokines and 18 chemokine receptors [78]. Chemokines are small proinflammatory chemoattractant cytokines that bind to specific G-protein-coupled seven-span transmembrane receptors. They are involved in cell activation, differentiation and trafficking. Chemokine receptors are defined by their ability to induce directional migration of cells toward a gradient of chemotactic cytokine [79; 80]. Human adipose tissue MSC (AT-MSCs) were found to secrete wide scale of cytokines, chemokines and growth factors. Human AT-MSCs express VEGF A, VEGFB, PDGF-BB, SCF, SDF-1α, HGF and CCL5, which may stimulate or inhibit the proliferation of tumor cells depending on the context [27; 48]. Breast tumor may be driven also by a population of cells that display stem cells properties. These cancer stem cells (CSC) mediate tumor metastasis and by virtue of their relative resistance to chemotherapy and radiation therapy, may contribute to treatment resistance and relapse following therapy [14]. These cells also interact with MSCs through cytokine network predominantly via IL6 and CXCL7 [81].

**CCL5/CCR5 axis.** CCL5 acts in a paracrine fashion on the tumor cells to enhance their motility, invasion and metastasis. It has an important role as a chemoattractant for stromal cells, such as macrophages, that express one of the receptors for CCL5, CCR5 [2]. We have observed increased CCL5 expression from AT-MSCs in response to A375 melanoma cells [48].

**VEGF/VEGFR1 (VEGFR2, NRP-1) axis.** VEGF regulates the formation of blood vessels and it is involved in endothelial cell physiology. The VEGF/VEGF-receptor axis is composed

of multiple ligands and receptors. VEGF binds to its cognate receptors VEGFR1, VEGFR2 and neutropilin 1 (NRP-1). Activation of the VEGF-receptor pathway triggers signalling processes that promote endothelial cell growth, proliferation, migration and survival [82; 83]. Synergistic increase in VEGF expression was identified in AT-MSCs/A375 cocultures and abrogation of VEGF/VEGFR signalling decreased AT-MSCs-promoted tumor xenotransplant growth [48].

**HGF/cMet axis.** HGF is primary expressed by fibroblasts. Breast tumor cells reprogram its surrounding fibroblasts to secrete HGF, in part, to support their own progression. Its cognate receptor c-Met is expressed by epithelial cells. HGF leads to an invasive phenotype, stimulates cell growth, influence cell scattering and, like TGF-β, can induce EMT in tumor cells. HGF is also known to promote angiogenesis *in vitro* and *in vivo.* Inducers of HGF expression are IL-1, bFGF, and PDGF [84], what is consistent with our findings of the cytokine secretion profile of AT-MSCs and subsequent increase in HGF upon coculture with tumor cells.

**EGF/EGFR/Akt pathway.** EGFR is a cell surface protein that is known to transmit extra-cellular mitogenic signalls, such as EGF and TGF-α through activating a number of downstream signaling cascades. The MAPK and PI3K/Akt signalling pathways downstream of EGF have been reported to play important roles in cancer cell proliferation and self-renewal. In breast cancer, overexpression of EGFR has been known to promote tumor survival and to suppress apoptosis [53; 85].

**SCF/c-Kit pathway.** The SCF/cKit pathway plays a crucial role during defined stages of embryogenesis and for the homeostasis of mature melanocytes. c-Kit has been implicated in a number of cancer forms in humans. c-Kit signals many cellular function such as cell survival, proliferation, differentiation, adhesion and chemotaxis (reviewed in [86]). We have found the expression of SCF in tumor cell and AT-MSCs, moreover, some breast cancer cell lines express cKit receptor, thus we conclude that this interaction also affect the final outcome. However, the role of this signalling axis in normal and malignant breast tissues remains to be further investigated [87].

**SDF-1α (CXCL12)/CXCR4 axis.** Stromal cell-derived factor-1α (SDF-1α, CXCL12) is a homeostatic chemokine. Constitutive secretion of CXCL12 by MSCs attracts tumor cells, acting through its cognate receptor, CXCR4. This receptor is involved in the spread and progression of tumor because it is essential for metastatic spread to organs where CXCL12 is expressed. CXCL12 itself can stimulate survival and growth of neoplastic cells in a paracrine fashion. Within hypoxic areas of tumors, both CXCL12 expression by fibroblasts and CXCR4 expression on tumor cells increases and stimulate tumor cell motility and invasiveness. CXCL12 from cancer/carcinoma-associated fibroblasts (CAFs) induces also recruitment of endothelial progenitors in endocrine fashion, which allows for tumor angiogenesis. CXCL12 gradient attracts CXCR4 positive tumor cells to bone marrow niches where marrow stromal cells secrete high levels of CXCL12. As a consequence, tumor cells can displace hametopoietc progenitor cells from their protective microenvironment, resulting in hematopoetic dysfunction [79; 80; 88]. Inhibition of SDF-1α/CXCR4 axis in vivo could prevent he AT-MSCs mediated abrogation of A375-tumor dormancy in our experiments *in vivo* [48]. Moreover, the study by Greco *et al.* suggested the role of this signalling axis in MSCs mediated chemoresistance in breast cancer tumor cells [54].

Tumor cells itself contribute to cytokine network by producing growth factors like fibroblastic growth factor (bFGF), VEGF, platelet-derived growth factor (PDGF), epidermal growth factor receptor (EGFR)-ligand and transforming growth factor β (TGF-β). TGF-β is responsible not only for shift from normal fibroblasts to activated tumor-associated fibroblasts, it also promotes single cell motility, which enables invasion into blood vessels In absence of TGF-β cells are restricted to collective movement and lymphatic spread [19]. All these cytokines are able to alter the normal tissue homeostasis stimulating the formation of new blood vessels as well as recruiting and activating immune cells and stromal fibroblasts [7].

## Conclusion

Tumor microenvironment represents a non-malignant part of the tumor organ which is composed of numerous cell types and extracellular compounds. These execute a complex network of signalling interactions which have important biological consequences for tumor behavior. Mesenchymal stromal cells can interact with the tumor cells and other components of the tumor stroma in numerous ways and according to our data their participation in tumors is predominantly dictated by the intrinsic tumor properties. Several potentially drugable signalling axes were identified within the tumor stroma thus providing indications how these can be exploited for the combination antitumor treatment.

Acknowledgments: The studies and experiments mentioned in this study were performed with the kind support provided by the League against Cancer, VEGA grant No. 2/0088/11, APVV grant No. APVV-0260-07 and APVV-0230-11.

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