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Review Article

The tendency of malignant transformation of mesenchymal stem cells in the inflammatory microenvironment, TAFs or TSCs?

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Abstract: MSCs showed broad application prospects in clinical medicine on account of those excellent characteristics. In inflammatory tumor microenvironment, MSCs can be recruited to nearly all the inflammatory sites and the mechanisms mainly be related to inflammatory cytokines, chemokines and other factors. In the development process of tumor, they showed dual effects of promotion or inhibition and the roles are still controversial. It is not known whether the graft show abnormal differentiation and proliferation or not. Not to mention how to control them differentiate into established directions. In this regard, most of the current studies are in vitro. At present, it is still not clear that whether MSCs in the inflammatory or tumoral microenvironment will undergo malignant transformation and even form tumors or not. We think that those roles of MSCs on tumors mainly depend on the changes of the microenvironment. When the microenvironment of the stem cells is changed, the interactions between both are changed too. Those complicated interactions may stimulate some key genes of signal pathways regulating proliferation, self-renewal, differentiation and other activities of MSCs. The primary coordination between stem cells and microenvironments is out of balance. So MSCs have more chance to get malignant changes. The occurrence of inflammatory microenvironment may lead to the trend of malignant transformation of MSCs: TSCs or TAFs. In conclusion, the tendency of malignant transformation of MSCs mainly depends on the interaction (cross-talk) between stem cells and microenvironment, especially the ability of stem cell to respond to circumstance changes and the ability to maintain stemness.

Keywords: MSCs, malignant transformation, TAF, TSC, inflammatory microenvironment

Introduction

Mesenchymal stem cells (MSCs) are some kind of adult stem cells characterized by multi-directional differentiate potentcy, which got a lot of attention at present. The most abundant MSCs is bone marrow mesenchymal stem cells (BMSCs). They have stronger self-proliferation and multi-directional differentiation potentials and are considered as ideal sources of seed cells in tissue engineering [1]. A lot of advantages such as convenience, without involving ethics, quick expansion, low immunogenicity and easy exogenous gene transfection and so on make them to be potential ideal carrier for gene therapy [2]. Deservedly they are widely applied in wound repairing, cell replacement therapy and gene therapies. However there are many problems to be solved prior to clinical studies such as the transplanting time, the composition and the number of transplanted cells, migration path of cells and survival time in host. Mostly important, it is not clear whether transplanted cell will bring new dangerous such as uncontrollable proliferation and differentiation or not. So the safety of MSCs is the primary factor limiting its clinical application. This has attracted more and more attention of scholars [3].

Stem cell microenvironment is composed of stem cells and related stromal cells, which can maintain the characteristics of stem cells and provides transient regeneration and differentia-

tion signals, thus keep the relative balance between stem cell self-renewal and differentiation. Once this balance is broken by some conditions such as continuous self-renewal resulting in the accumulation of gene mutations, then it is possible that cancer occurs [4]. Inflammation is closely related to tumor. Clarifying the tendency of malignant transformation of MSCs in the inflammatory microenvironment and the correlation of abnormal circumstances and malignant transformation will help people to avoid the dangerous factors promoting malignant transformation of MSCs before transplanting.

Below, we will separately discuss the interaction between MSCs and inflammatory microenvironment and the tendency of malignant transformation of MSCs.

The interaction between MSCs and inflammatory tumor microenvironment

Inflammatory tumor microenvironment recruits MSCs homing

In the inflammatory tumor microenvironment, MSCs can be recruited to nearly all the damaged tissues such as ischemic myocardial tissue, region of damaged skin and gastrointestinal mucous membrane after radiotherapy [4]. The innate tendency of MSCs migrating to the tumor site has been demonstrated in glioma, breast, colon and lung cancer in murine xenograft models. In these models, MSCs has been successfully traced through a variety of different injection routes, including the carotid artery, the rat tail vein, the femur, tibia and other migration to the tumor tissue phenomenon. MSCs in mouse tumor tissues might be from bone marrow MSCs. In other words, the MSCs could be through the circulation of the blood. Adipose tissues near tumors provide another source of MSCs in tumors [5].

The homing mechanisms that microenvironments inducing MSCs is mainly depend on a variety of inflammatory cytokine (TNF-α, IFN-γ, IL-1β, IL-6, IL-8) and chemokines (SDF-1/CXCR4, MMP, VCAM-1, CXCL, GRO-a, MCP-1) with multiple functions, growth factors (TGF-β, PGF, PDGF, HGF) and other factors (HIF-1, LL-37) [4]. Some circulating bone marrow cells stimulated by inflammatory signals adhere to vascular endothelial cells. After deformation, they penetrate from the vessel wall and migrate to the tissue damage site or the tumor site. Then MSCs participate in the formation of tumor stroma, thereby regulating the microenvironment of the tumor [6, 7].

TNF-α with a high expression level in tumor tissues can upregulate expression levels of vascular cell adhesion molecule-1 (VCAM-1) on MSCs. It facilitates the adhesion of MSCs to endothelial cells [8]. IL-6 with a high expression level in tumor tissues could directly induce MSC expressing IL-6 receptors in these tissues. In addition, large quantities of SDF-1 secreted by tumor cells could induce MSCs expressing the SDF-1 receptor CXC chemokine receptor 4 (CXCR4) migrations [9].

On the whole, the homing mechanisms are mainly depending on a variety of inflammatory cytokines and chemokines with multiple functions. In the tumor microenvironment, tumor cells and inflammatory cells secrete various cytokines. These cytokines can promote MSCs migrate to the tumor site and participate in the formation of tumor stroma, thereby regulating the microenvironment (**Figure 1**).

MSCs participate in the inflammatory tumor microenvironment

The occurrence and evolution of tumor is a complex process involved in regulations of multi-steps, multi-stages and multi-factors. The tumor is not a self-sufficient entity, but is composed of two parts, the tumor parenchyma and stroma. Macrophages, endothelial cells, lymphocytes, neutrophils, fibroblasts and peripheral cells were all found in the tumor stroma [8]. These cells participate in the regulation of the tumor microenvironment by secreting hormones, cytokines, chemokines, and proteases. Tumor microenvironment plays a very important role in the growth, invasion and metastasis of tumor.

The inflammatory tumor microenvironment includes the inflammatory factors (TNF-α, IFN-γ, Il-8, Il-1, TGF-β), growth factor (HGF, VEGF, PDGF), chemokine (SDF-1) and other factors (MMP). The major non-tumor cells include inflammatory cells such as lymphocytes and macrophages, bone marrow derived suppressor cells, endothelial cells, TAF and MSCs [4, 10] (**Figure 2**).

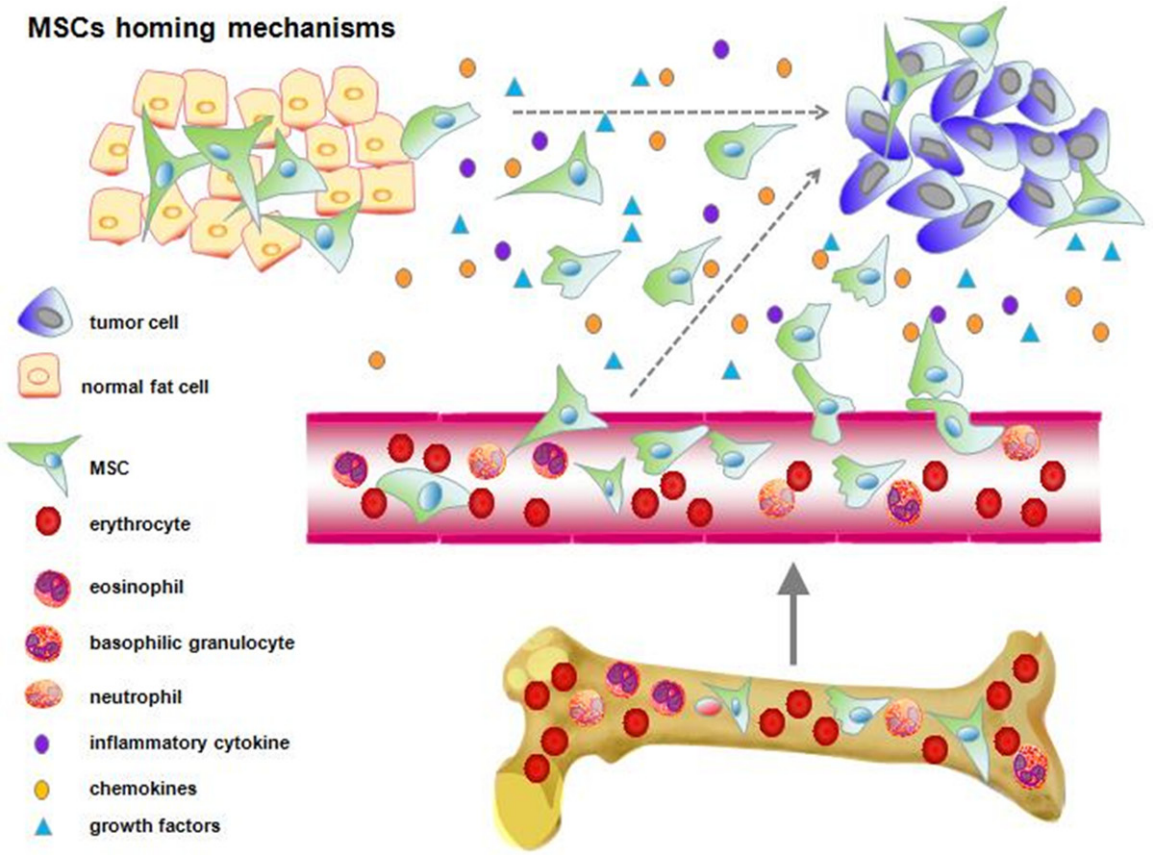

Figure 1. The homing mechanisms of MSCs. MSCs could be through the circulation of the blood. Adipose tissues near tumors provide another source of MSCs in tumors. The homing mechanisms is mainly depend on a variety of inflammatory cytokines and chemokines with multiple functions, growth factors and other factors. Some circulating bone marrow cells stimulated by inflammatory signals adhere to vascular endothelial cells. After deformation, they penetrate from the vessel wall and migrate to the tissue damage site. Those cytokines secreted by various cells can promote MSCs migrate to the tumor site and participate in the formation of tumor stroma, thereby regulating tumor microenvironment.

Inflammatory factors promote malignant transformation of MSCs

As an important component of the inflammatory microenvironment, inflammatory factor is closely related to the malignant transformation of stem cells. It is reported that the inflammatory factor IL-6 can make the non-tumor stem cells transform into cancer stem cells [11]. It is believed that IL-6 and IL-1 play an important role in cell malignant transformation and tumor progression [12]. As a key transcription factor of these factors, NF-kappa B is considered an important bridge to link inflammation and tumor initiation and development. The experimental results show that the inflammation caused by NF-kappa B is essential for the malignant transformation of normal breast cells and the maintenance of the phenotype of tumor stem cells [13]. NF-kappa B may regulate the stemness of TSCs by mediating the expression of cytokines. In fact, many of the factors in the tumor microenvironment are able to activate the NF-kappa B signaling pathway. The inflammatory reaction not only regulates the self-renewal and differentiation of stem cells, but also increases the probability of mutation and enhance the proliferating ability of mutant cells.

The diversified effects of MSCs on tumorigenesis

In the process of tumor development, MSCs showed dual effects of promotion and inhibition [14]. Study have demonstrated MSCs pretreated with TLR4 can produce proinflammatory mediators and inhibit growth and metastasis

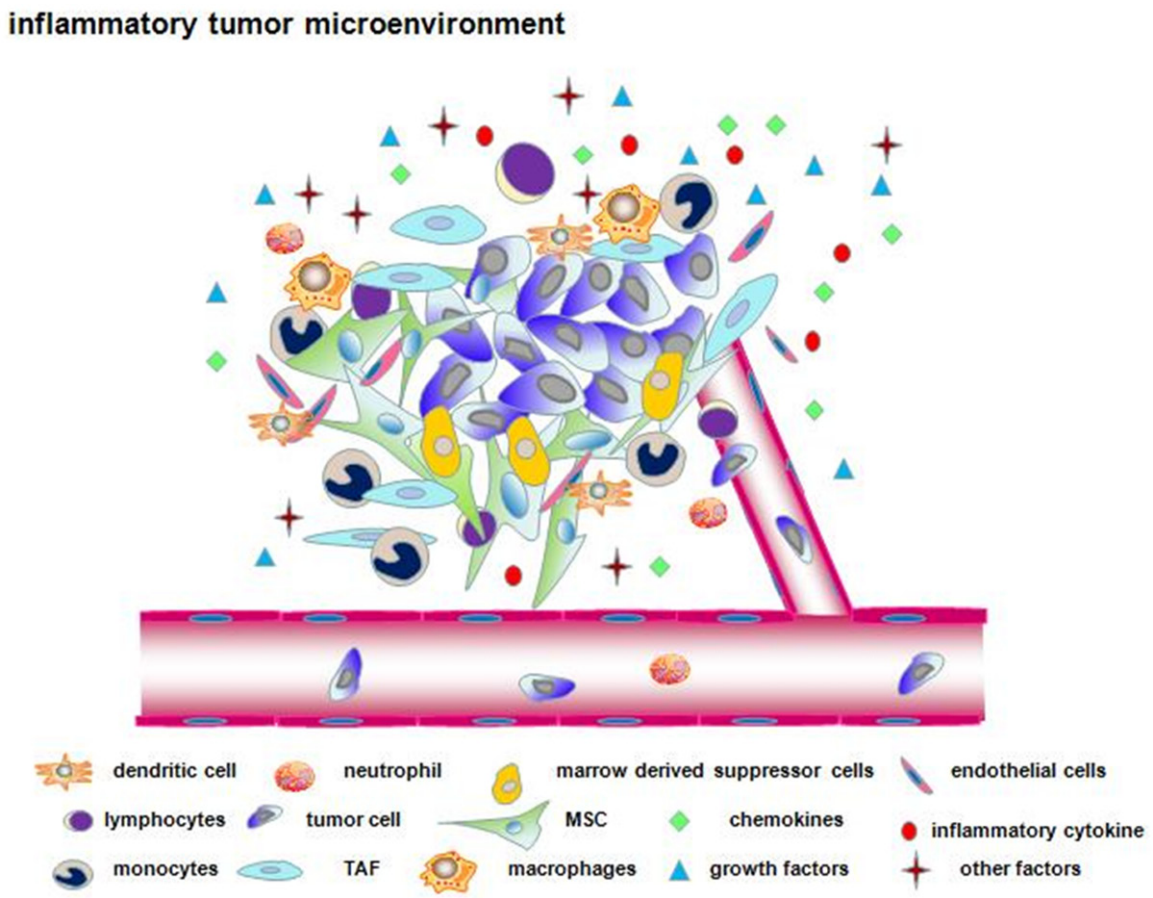

Figure 2. Inflammatory tumor microenvironment. The inflammatory tumor microenvironment includes the inflammatory factors, growth factors, chemokines and other factors. The major non-tumor cells include inflammatory cells such as lymphocytes and macrophages, bone marrow derived suppressor cells, endothelial cells, TAF and MSCs.

of tumor, however, MSCs pretreated with TLR3 can produce active factors with immunosuppressive effects and play an important role in the progress of tumor metastasis [15]. There are many factors affecting the function of MSCs, such as the low oxygen condition, extracellular matrix components, extracellular acidic environment, inflammatory mediators and so on. When MSCs reaches the tumor site, its role of promoting or inhibiting the growth of the tumor mainly depends on the changes of the tumor microenvironment.

Effects of MSCs inhibiting the development of tumor

Used for gene targeted therapy of cancer: MSCs are easy to isolate and amplify in vitro, and they have good abilities of migration, tumor tropism and low immunity. It is easy to import foreign genes. So MSCs are becoming the first choice for tumor biological therapy [7]. As a tumor targeted gene therapy vectors, MSCs modified with gene can induce tumor cell apoptosis and enhance anti-tumor effects. However, due to limited gene transduction technology, safety of genetically modified engineering and the effectiveness of transporting various therapeutic cytokine and so on, a lot of work must be done before clinical application [16].

Inhibition of tumor growth and invasion, metastasis: There are some studies suggested that MSCs could reduce tumor metastasis after transplanting them into model animals. BMSCs in Kaposi sarcoma can play an anti-tumor role through inactivated Akt pathway. MSCs blocked the Akt signal so as to regulate the tumor cell cycle and inhibit its proliferation. MSCs also can upregulate the miRNA expression levels of the cell cycle negative regulation factor p21 and apoptosis related protease caspase-3, and arrest the cell in the G0/G1 phase to inhibit the proliferation of tumor cells. MSCs inhibits the

growth of human glioma by inhibiting angiogenesis [17].

Effects of MSCs promoting the development of tumor

Now scholars are beginning to pay more and more attention to the role of MSCs in the development process of tumor. MSCs can promote tumor cell growth and proliferation, enhance capabilities of invasion and metastasis, participate in the epithelial-mesenchymal transformation (EMT) and suppress the immune response [18, 19].

MSCs promote tumor cell growth, proliferation, invasion and metastasis: Study reported that the proliferation and metastasis of cancer cells can be enhanced after subcutaneous transplantation of cancer cells and MSCs [20]. MSCs not only promotes the growth of the tumor, but also increases the possibility of metastasis of tumor. Tumor metastasis was enhanced in mouse fat pad tumor metastasis model with tail vein injection of MSCs. Metastatic nodules were found in the half of experimental group, while metastatic nodules were found in only 17% control group [21]. MSCs were able to migrate to primary tumor site and metastasis site of colon carcinoma after intravenous injection. After cotransplantation MSCs with colon cancer cells into the spleen, cancer was transferred to the liver quickly [22].

A series of signaling molecules secreted by MSCs were involved in tumor metastasis such as CCL5 [23], Hypoxia inducible factor (HIF) [24], Matrix metallo proteinases (MMPs) [20, 24], miRNA [25], IL-6 [26] and so on [27].

MSCs participate in the EMT: MSCs can participate in EMT and promote tumor formation and development [28]. Its possible mechanisms included the activation of TGF-β signaling pathway, the releasing of ILs and the interaction between CXCL16 and its receptor, and so on. MSCs can affect the occurrence of EMT in liver cancer cells by secreting TGF-β, which enhance the ability of invasion and metastasis of carcinoma cells [29]. The activation of Toll-like receptor 4 (TLR4) on MSCs cell surface and lipopolysaccharide (LPS) can induce MSCs to secrete of a large variety of proinflammatory mediators including IL-17, IL-3, monokine induced by interferon gamma (MIG) and so on. These cytokines can promote EMT and malignant tumor metastasis. Prostate cancer cells derived from human and mouse both can secrete CXCL16, and the MSCs expresses of CXCL16 receptor CX-CR6. Combination of the two will promote MSCs to differentiate into fibroblasts activated by tumor cells. Fibroblasts secrete a large number of SDF-1a to induce EMT of tumor cells. Then promote tumor cells to infiltrate other organs [30].

MSCs suppress the immune response: MSCs are able to suppress the immune response. That's to say, they can form a local immunosuppressive microenvironment where proliferation and activation of all kinds of main immune cells would be restrained through the low immune phenotype, secrete anti-inflammatory factor and so on a variety of mechanisms [31]. So it is good for tumor growth and immune escape.

So mesenchymal stem cells can promote tumor cell growth and proliferation, enhance capabilities of invasion and metastasis, participate in the EMT and suppress the immune response (**Figure 3**). We think that the influence of MSC on tumor mainly depends on living environment of MSCs.

Malignant transformation tendency of MSCs in the inflammatory microenvironment

Theoretically speaking, compared with the matured cells, stem cells can divide and proliferate continuously, so they have more chances to get the mutant gene inheritance to the progeny cells resulting in the accumulation of the genetic variation and malignant transformation of stem cells. However the matured tissue cells cannot accumulatee the number of mutated genes required for malignant transformation due to short survival time [32]. MSCs can reached all over the body through the blood circulation, and across the capillaries into the site of damaged tissue in sample amoebic movement way, which are involved in tissue injury repairing [33]. And repeated tissue injury, inflammation and regeneration are closely related to the occurrence of tumor. So the space conditions for the occurrence of malignant transformation are provided because of the characteristics of MSCs homing to the site of inflammation.

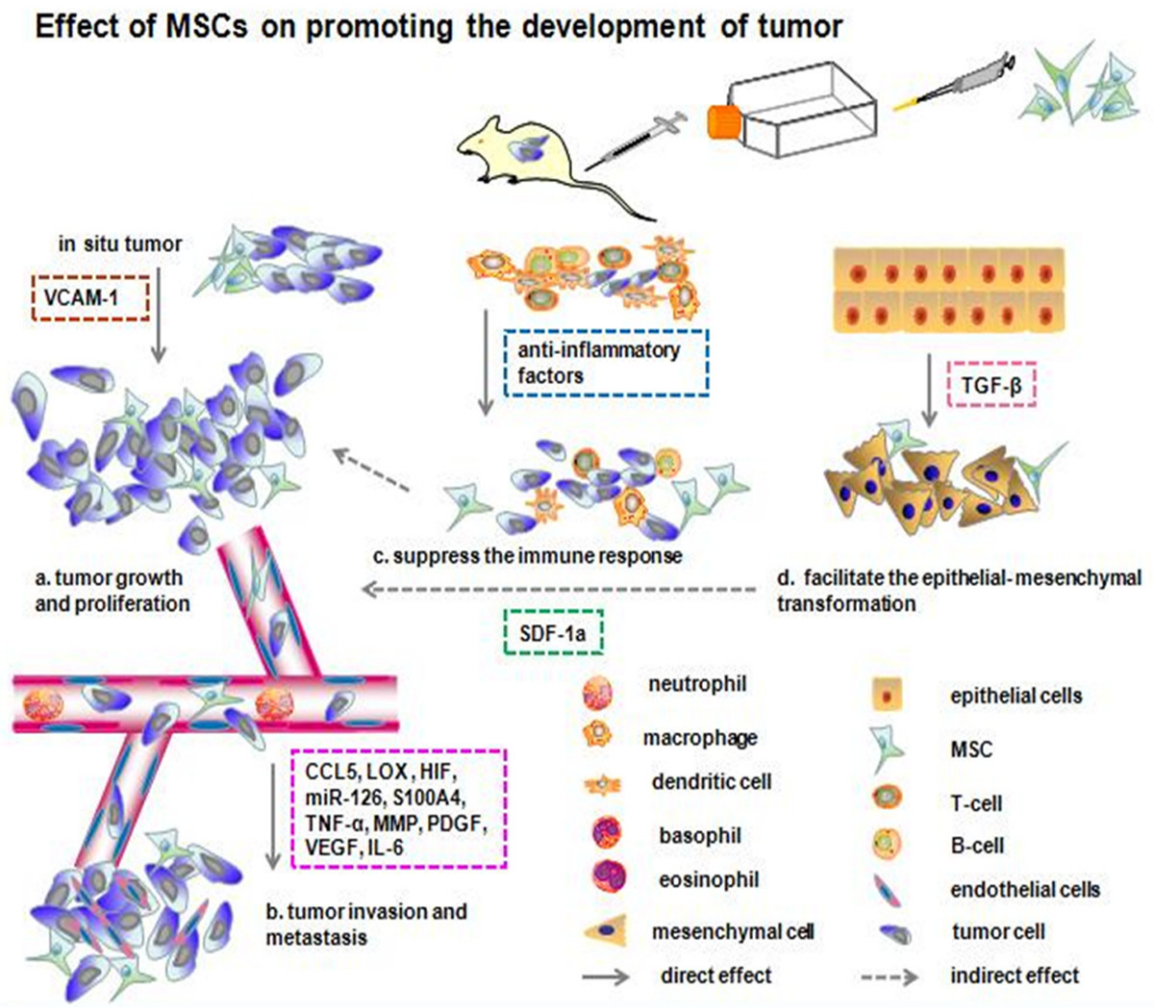

Figure 3. MSCs promote development of tumor. Mesenchymal stem cells can promote tumor cell growth and proliferation, enhance capabilities of invasion and metastasis, participate in the epithelial-mesenchymal transformation and suppress the immune response.

With the further research, some scholars have reported that the stem cells will spontaneously transform malignantly after long-term culturing. Cancer occurred after transplantation MSCs into the animal body, before this MSCs have been cultured for less than 8 months (division 90~140 times) [34]. BMSCs can undergo spontaneously malignant transformation in vitro culturing for a long times, it would be a serious hazard for tissue engineering applications. The probability of malignant transformation of BMSCs in vitro long-term training is 45.8%. The morphology and phenotype of transformed cells are changed, the abilities of differentiation is lost, also soft AGAR clone formation test result is positive. Cells have strong tumorigenicity so that tumor was found rapidly after they were inoculated into nude mice [35]. There are also some research report that normal adult stem cells may undergo malignant transformation under the carcinogen arsenic, and form the malignant cells such as cancer stem cell [36]. Experiments showed the malignant transformation of BMSC induced by IL-4, GM-CSF in vitro conditions, and deteriorating cells could develop into a tumor [37].

Malignant transformation of MSCs may be the source of tumor stem cells (TSCs) or tumor associated fibroblasts (TAFs).

Tumor stem cell (TSCs)

The current studies on human solid tumors found that not all tumor cells have the same tumorigenicity. Reya *et al*. [38] put forward the

hypothesis of "tumor stem cell" based on the similarily biological characteristics between stem cells and some tumor cells. It is believed that there are very few tumor stem cells with self renewal, unlimited proliferation and multi differentiation potential in tumor tissues. They are the specific cells that contribute to the occurrence and development of the tumor. After that, some new contents on the origin, pattern and clinical diagnosis and treatment of the tumor enriched the theory [39].

The theory that tumor stem cell is the initiating cell of tumor is accepted by more and more scholars, but origin of cancer stem cell is still not determined. At present, there are two main hypotheses: (1) The mutation of normal stem cells derived from the corresponding tissues [40]. (2) Some of the original cells that have begun to differentiate or matured cells re-obtain self-renewal capacity before the carcinogenesis, lose the ability to differentiate, then evolved into tumor stem cell [41]. The former is more recognized, because the molecular phenotype of differentiation markers is consistent between tumor stem cells identified and the normal stem cell in the corresponding organ. Also stem cells have the same unlimited proliferative capacity as tumor and they have the chance of multiple mutations causing tumor.

Increasing researches about stem cell have indicated that tumor can originate from the abnormal differentiation of stem cells. BMSCs occurred malignant transformation may be one of the sources of cancer stem cells [42]. The occurrence of tumor stem cells is likely closely related with mutations in the process of long-term self-renewal. Once some signal pathways regulating proliferation and differentiation change, the corresponding biological events of stem cell will be disturbed by those abnormal signals. Then stem cells undergoing malignant transformation and excessive proliferation developed into cancer. Stem cells are often the target cells of malignant transformation.

Normal stem cell requires the surrounding microenvironment to maintain cellular functions. Extracellular matrix is an important component in the microenvironment of tissue, which provides the key biochemical and physical signals for the initiation and maintenance of cell function, so as to regulate the balance between self-renewal and differentiation. Stem cell microenvironment is composed of cells, adhesion molecules, signaling molecules and extracellular matrix. It has impoortant roles of maintaining the self-renewal of stem cells, regulating the balance of self-renewal and differentiation, adjusting the proliferation, and thus it can affect the occurrence of cancer. Different stem cells have different microenvironment, which can affect the abnormal differentiation of stem cells and even the formation of tumor [10]. Some damaging factors may also be carcinogenic factors, local inflammatory microenvironment have a direct or indirect impact on MSCs. Stem cells are more sensitive to external carcinogens than matured cells, so they are more likely to undergo malignant transformation. Abnormal signal pathway may lead to abnormal differentiation of stem cells.

Due to the destruction of the microenvironment, the signal molecules inducing the maturation and differentiation of cells appear abnormal or are unable to effect on the targeted cells. The proliferated cells can not differentiate to mature cells, and the total number of functional cells is reduced. The organism continuously generates the stimulating proliferation signals, which promote the immature cells to be in the state of proliferation unceasingly, then malignant proliferation occurs [43]. Experiments have confirmed that inflammatory factors such as IFN-γ, TNF-α can lead to the decline of functions of self-renewal and differentiation on MSC, which may increase the cellular susceptibility to malignant transformation through the NF-nuclear factor kappa B/SMAD7 signaling pathway [44]. These results reveal the abnormal changes regulating proliferation signal pathway may be the molecular basis of malignant transformation.

Tumor-associated fibroblasts (TAFs)

TAFs are important components of the tumor microenvironment. TAFs have the role of enhancing tumor angiogenesis and promoting tumor growth, invasion and metastasis. They promote the proliferation of tumor cells by secreting a variety of growth factors in the tumor cells. Usually there are 4 aspects defining TAF-like phenotype: ① fibroblast activation markers including fibroblast specific protein (FSP) and fibroblast activation protein (FAP); ② the invasive markers including thrombospon-

din-1, tenascin-C and matrilysin; ③ production of vascular marker proteins (α-SMA, VEGF-AA); ④ growth factors and inflammatory factors promoting the tumor growth including EGF, HGF, IL-6, B-FGF [45]. TAF is mainly derived from: ① Transfer of fibroblast ② Transfer of MSCs derived from bone marrow by the circulation ③ Primary tissue stem cell differentiation ④ epithelial mesenchymal transition.

TAFs have a lot of similarities with MSCs. Many studies found that MSCs is an important source of TAFs [46]. Models of inflammation-induced gastric cancer indicated that at least 20% of TAFs originated from BMSCs [45]. MSCs subjected to signal molecule in the tumor microenvironment stimulation for a period of time were detected the phenotype of fibroblasts, which are generally considered to be TAFs. KIDD S found that subpopulations of TAFs were recruited from two distinct sources [5]. The majority of TAFs (FSP positive and FAP positive) originate from BMSCs, whereas most vascular and fibrovascular stroma (pericytes, α-SMA positive myofibroblasts, and endothelial cells) originates from neighboring adipose tissue. Zhu Wei et al. [47] confirmed that tumor microenvironment induced human BMSCs would be activated and differentiated into TAFs, which play a role in promoting tumor growth.

So, the formation, the relocation and even the cellular function of TAFs derived from BMSCs are closely related to the occurrence and development of tumors. Also, these results highlight the capacity for tumors to utilize multiple sources of structural cells in a systematic and discriminative manner [5].

A large number of studies have found that MSCs, as precursor cells, may participate in the composition of the cell matrix in the tumor microenvironment in the form of TAFs. TAFs can secrete plenty of active factors. There are mainly 4 kinds: (1) growth factors: EGF, HGF, b-FGF, TGF-β, insulin-like growth factor (IGF); (2) cytokines: IL-6; (3) chemokines: SDF-1; (4) proteases: MMPs. TAFs can increase the secretion of VEGF, levels of which is 5 times of MSCs [48]. VEGF may play an important role in regulation of angiogenesis. It is essential for cancer to maintain growth and metastasis. Except for soluble factors, TAFs may secrete some proteins to facilitate tumors growth; however the research is not much.

BMSCs transferred to the tumor site were mediated to differentiate into TAFs through the TGF-β/Smad signaling pathway. Study showed that the tumor cells exosomes vesicles contained TGF-β and it can be combined with the type-I TGF receptor on the MSCs to induce Smad2/3 and P38 activation, so MSCs differentiate to TAFs [49]. Bone morphogenetic protein and activin membrane-bound inhibitor (BAMBI) gene was reproduced to BMSCs by viral vector, and the BAMBI protein was synthesized. It is a pseudo receptor of TGF-β and very similar with the extracellular domain of type-I TGF receptor. So it can compete with type I receptor to bind with the ligand, and the formation of functional ligand -receptor complexes is hindered. Lacking of intracellular serine/threonine kinase domain of type-I receptor, BAMBI have no serine/threonine kinase activity and cannot phosphorylate Smads of downstream so that the TGF-β/Smad signal transmission was blocked. MSCs reproduced encoding BAMBI gene was inhibited to transformed to TAFs and the effect of promoting tumor growth was also disappeared [50] (**Figure 4**).

TGF-β from exosomes vesicles of ovarian cancer cells can induce MSCs to differentiate into TAFs. The phosphorylation of Smad2 increased during this process. In addition, the HOXA9 gene of epithelial ovarian cancer cell can control the transcription level of TGF-β and increase the expression of TGF-β in the matrix thus facilitate MSCs to differentiate into TAFs [51]. Note that the mechanism of differentiation influences MSCs alone, but will not affect the tumor growth, metastasis and the properties of MSCs such as tropism and stemness [52].

Changes in genetic stability

Of course, some scholars believe that BMSCs in vitro amplification culture does not increase the risk of malignant transformation, so MSCs are not prone to spontaneous malignant transformation [53]. However, MSCs can be induced by certain chemical agents such as 3-MCA, 1-oxygen-4-nitro quinoline and so on. The mechanism of the malignant transformation of BMSCs is related to chromosomal abnormalities, the increase of telomerase activity and the decrease or loss of P53 function. The evaluation on biological safety of BMSCs should be more stringent before clinical application [54].

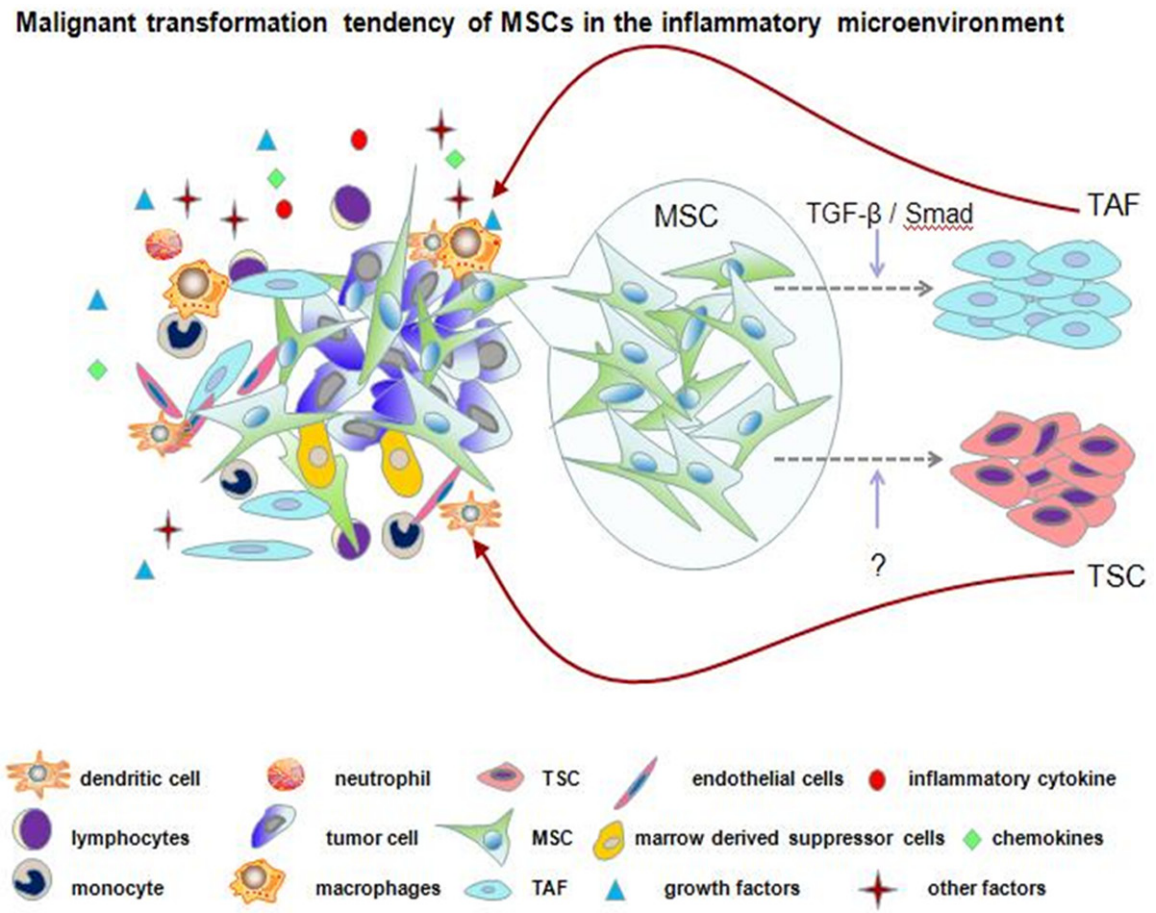

Figure 4. Malignant transformation tendency of MSCs in inflammatory microenvironment. The differentiation of MSCs into TAFs can be completed through the TGF-β/Smad signal pathways. The mechanism of differentiation influences MSCs alone, but will not affect the tumor growth, metastasis and the properties of MSCs.

Possible signaling pathways involved in the regulation of malignant transformation

Tissue microenvironment contains many inflammatory cytokines, which may regulate the related genes and their products involved in differentiation and renewal of stem cell, which lead to malignant transformation of stem cells.

At present, there are many signal transduction pathways related to growth and regulation mechanism of stem cell such as Wnt/β-catenin (involved in stem cell self-renewal and tumor formation), SHH (sonic hedgehog, involved in stem cell self-renewal), Notch (transfer cell differentiation inhibitory signal), TGF-β/Smad pathways and IL-6/STAT3 pathways and so on (**Table 1**, [55]).

NF-κB/IL6/STAT3 signaling pathway

Inflammatory process associated with the activation of oncogenes can lead to amplification of nuclear factor-kappa B/IL-6/STAT3 cascade, which accelerate the occurrence or progression of the tumor. This is also a strong evidence of the effect of inflammation on the proliferation of cancer cells [56]. Because of its close relationship with the tumor, STAT3 is getting more and more attention. The protein family transducer and activator of transcription are a group of related proteins that can be activated by different cytokines receptors. It is the focus of EGFR, IL-6/JAK, Src and other multiple oncogenic tyrosine kinase signaling pathways. STAT3 is found in a variety of tumor cells with persistent excessive activation. Excessive activation of STAT3 induces a high expression of key genes that are closely associated with cell proliferation, differentiation and apoptosis. It promotes cell proliferation, malignant transformation and blocking cell apoptosis through a variety of ways. It also plays a significant role in the self-renewal and proliferation of tumor stem cells [57].

Table 1. KEGG pathway analysis of the related genes in MSCs malignant transformation

Pathway	Gene number	Bayes factor	Gene
Toll-like receptor signaling	19	15.41	*Fos*, *Ifna1*, *Ifna12*, *Ifna14*, *Ifna5*, *Ifna6*, *Ifna7*, *Ifna9*, *Ifnab*, *Ikbkb*, *Il6*, *LOC242517*, *Ly96*, *Mapk14*, *Nfkb1*, *Nfkb2*, *Pik3ca*, *Stat1*, *Tnf*
Cell cycle	18	14.66	*ABL1*, *CCNB1*, *CCND1*, *CCND2*, *CDC2*, *CDK2*, *CDK4*, *CDK6*, *CDKN1A*, *CDKN2A*, *GSK3B*, *HDAC1*, *HDAC2*, *PRKDC*, *RB1*, *MAD3*, *TGFB1*, *TP53*
Focal adhesion	27	13.31	*ABL1*, *BRAF*, *CCND1*, *CCND2*, *COL1A1*, *COL1A2*, *COL5A2*, *CTNNB1*, *EGF*, *EGFR*, *GSK3B*, *HRAS*, *IGF1*, *ITGAV*, *MAPK1*, *MAPK3*, *PDGFB*, *PDGFC*, *PDGFD*, *PDGFRB*, *PIK3CA*, *PTEN*, *PTK2*, *RHOA*, *SRC*, *TNC*, *VEGF*
Cytokine-cytokine receptor interaction	27	10.32	*Ccl12*, *Csf2*, *Cxcl1*, *Cxcl12*, *Cxcl5*, *Egf*, *Egfr*, *Ifna1*, *Ifna12*, *Ifna14*, *Ifna5*, *Ifna6*, *Ifna7*, *Ifna9*, *Ifnab*, *Il4*, *Il6*, *Il6st*, *LOC242517*, *Pdgfa*, *Pdgfb*, *Pdgfc*, *Pdgfd*, *Pdgfrb*, *Tgfb1*, *Tnf*, *Vegfa*
Gap junction	15	9.65	*Cdc2a*, *Csnk1a1*, *Egf*, *Egfr*, *Hras1*, *Map2k2*, *Mapk1*, *Mapk3*, *Pdgfa*, *Pdgfb*, *Pdgfc*, *Pdgfd*, *Pdgfrb*, *Src*, *Tjp1*
Wnt signaling	15	8.44	*Apc*, *Axin1*, *Axin2*, *Catnb*, *Ccnd1*, *Csnk1a1*, *Fosl1*, *Gsk3b*, *Madh2*, *Madh3*, *Myc*, *Rhoa*, *Sfrp1*, *Tp53*, *Wnt3*
Adherens junction	12	6.77	*CDH1*, *CTNNB1*, *EGFR*, *MAPK1*, *MAPK3*, *RHOA*, *SMAD2*, *SMAD3*, *SNAI1*, *SNAI2*, *SRC*, *TJP1*
MAPK signaling	23	6.12	*Braf*, *Casp3*, *Ddit3*, *Egf*, *Egfr*, *Fgf2*, *Fgf7*, *Fos*, *Hras1*, *Ikbkb*, *Map2k2*, *Mapk1*, *Mapk14*, *Mapk3*, *Myc*, *Nfkb1*, *Nfkb2*, *Pdgfa*, *Pdgfb*, *Pdgfrb*, *Tgfb1*, *Tnf*, *Trp53*
TGF-beta signaling	12	5.76	*BMP4*, *MAPK1*, *MAPK3*, *MYC*, *NODAL*, *RHOA*, *SMAD2*, *SMAD3*, *SMAD7*, *TGFB1*, *TNF*, *ZFYVE9*
Neuroactive ligand-receptor interaction	2	3.23	*C5r1*, *Nmb*
Tight junction	11	1.78	*CDK4*, *CLDN1*, *CTNNB1*, *HCLS1*, *HRAS*, *OCLN*, *PTEN*, *RHOA*, *SRC*, *TJP1*, *TJP3*
Regulation of actin cytoskeleton	16	1.52	*APC*, *BRAF*, *EGF*, *EGFR*, *FGF2*, *FGF7*, *HRAS*, *ITGAV*, *MAP2K2*, *MAPK1*, *MAPK3*, *PDGFB*, *PDGFRB*, *PIK3CA*, *PTK2*, *RHOA*
Jak-STAT signaling pathway	13	1.29	*CCND1*, *CCND2*, *CSF2*, *IFNA1*, *IFNA13*, *IL4*, *IL6*, *IL6ST*, *JAK1*, *MYC*, *PIK3CA*, *STAT1*, *STAT3*
Hedgehog signaling pathway	7	1.14	*Bmp4*, *Csnk1a1*, *Gli1*, *Gsk3b*, *Ptch1*, *Wnt3*, *Wnt3a*

TGF-β/Smad signaling pathway

TGF-β is a member of the transforming growth factor family. As a more efficient growth factor, TGF-β can stimulate the secretion and deposition of extracellular matrix. It plays an important role in the induction of apoptosis, angiogenesis, cell cycle, cell migration and so on. TGF-β transmits signal mainly through the Smad pathway, and its signal transduction pathway by which it plays a variety of biological effects in stem cells has a wide range of cross talk with other signaling pathways such as MAPK, p38, JNK, PI3K/Akt signal [58].

Tumor necrosis factor alpha (TNF-α)/TNFR signaling pathway

TNF-α is a cytokine depended on the activation of macrophages/monocytes and has biological multi-effects. Tumor necrosis factor alpha plays a key role in many physiological and pathological processes, such as inflammatory response, cellular immunity, tumor immunity and so on. The biological effects of TNF are dependent on 2 types TNF receptor (TNFR) on the cell surface. The signal transduction pathways of TNF mainly include caspase family mediating apoptosis, and the activation of the transcription factor NF-kappa B and JNK protein kinase, which is mediated by adaptor protein TRAF. Motif of TNFR2 combinates to TRAF. The complex can activate NF-κB and JNK kinase, induce gene transcription and promote cell survival, proliferation and differentiation. In the absence of TNF, NF-κB binds with inhibitory subunit I nuclear factor kappa B (IκB) and masks the nuclear localization signal (NLS) of NF-nuclear factor kappa B, consequently, NF-nuclear factor kappa B is remained in the cytoplasm.TNF expose NLS of NF-κB through the IKK kinase complex, so that NF-κB enters the nuclear transcription, inducing a variety of apoptosis inhibitory factor expression. The key step of activation is the transferation of NF-κB from cytoplasm to nucleus [59].

Discussion

In summary, the correlation between the inflammatory microenvironment and the malignant transformation of mesenchymal stem cells has

been more and more recognized, but its specific mechanism has not been fully clarified. The existing research in vitro mainly focused on the circumstances in which tumor and mesenchymal stem cells are co-cultured directly or indirectly to explore the effects of tumor inflammatory microenvironment on mesenchymal stem cells. Some of the important related signaling pathways and inflammatory factors have been found to play an important role during the process. The long-term stimulation of inflammatory microenvironment in vivo has not been reported. The possible reason is that, as a whole, the body's response to inflammatory reaction and related immune response is more complicated and difficult to explain.

We believe that the tendency of malignant transformation of mesenchymal stem cells in the inflammatory microenvironment is not only affected by the micro environment changes, but it mainly depends on the interaction between stem cells and microenvironment (cross-talk), the ability of stem cell to respond to circumstance changes and the ability to maintain stemness. The related work is being carried out. We imitate inflammatory microenvironment with some inflammatory factors and observe the malignant transformation tendency of mesenchymal stem cells and the imbalance of inflammatory signal pathway. It is expected to reveal the mechanism of the major signaling pathways that mediate malignant transformation. This will help us to avoid the inducing factors that promote the malignant transformation of the mesenchymal stem cells, so as to improve the safety of the clinical application of mesenchymal stem cells.

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Disclosure of conflict of interest

None.

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