CANCER STEM CELL BIOLOGY and CANCER VACCINES
(5)

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Mesenchymal Stem Cell
Cancer Cell
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Mesenchymal Stem Cells:

- Cells capable to differentiate into mesodermal-derived tissues, such as adipocytes, chondrocytes, and osteoblasts, are called mesenchymal stem cells (MSCs) and they are suggested to reside in all human organs and tissues.

- MSC can circulate in the peripheral blood and are detected in tissues other than bone marrow, such as subcutaneous fat, periodontal ligament, umbilical cord blood, fetal tissues, lymph nodes, and adult spleen and thymus, thus hypothesizing a “mesenchymal organization,” virtually present in all post-natal organs and tissues.

- Some reports describe that MSCs can also differentiate in non-mesodermal cell types, such as gut and skin epithelial cells, hepatocytes, pneumocytes, and neuronals.

Mesenchymal Stem Cells:

- However, there is a lack of accuracy regarding to both terminology and biological characteristics.

- Many authors state that MSCs are considered different from so-called multipotent adult progenitor cells that are able to differentiate into neurons, epithelial cells, as well as in cells of mesenchymal origin.

- Another typology of stem cells, different from MSCs, are multipotent mesenchymal stromal cells from which derive only cells belonging to mesodermal tissues, such as fat, muscle, bone, and cartilage cells.

- Such differences both in terminology and biological characteristics home probably in the variability of experimental methodologies, rather than in the existence of different stem cells of mesenchymal origin, although it is possible to hypothesize that it can exist a gradient of MSC differentiation as well as demonstrated for hematopoietic stem cell precursors.

Mesenchymal Stem Cells:

- Non-haematopoietic stem cells that have generated a significant amount of interest as a result of their apparent ability to home to the tumour site following systemic delivery. MSCs have an inherent ability both to self-renew and to differentiate into multiple lineages including osteoblasts, chondrocytes and adipocytes.

- Expression of CD105, CD73 and CD90 in >95% of the culture,

  Absence of CD14, CD34, CD45, CD19, HLA-DR and CD4.

- When introduced systemically to healthy animals, MSCs have been shown to home preferentially to the lung, liver and bone, and were found to a lesser extent in other tissues.

- Engineered MSCs may target multiple tumor types; because they are considered immunoprivileged, possibly due to low expression of Ag(HLA) MHC class 1, and no expression of CD40, CD80 and CD86.

Mesenchymal Stem Cells:

- MSCs are rare with $1/10^5$ cells in bone marrow and lose their differentiation potential after 40 doublings.

- These cells are also able to migrate from the circulation to different tissues in response to a variety of signals. This process is called “homing” and is regulated by a specific pattern of chemokines and chemokine’s receptors.

- MSCs are recruited to the site of wound healing to repair injured tissues in a similar process than the one found in tumors.

Tumor tropism of MSCs

- Tumour-specific migration of MSCs appears to be dependent upon the biological properties of the tumour microenvironment.

- Integration of MSCs into the tumour stroma is thought to be mediated by high local concentrations of inflammatory chemokines and growth factors.

- The tumour microenvironment is considered a site of chronic inflammation . This environment may mediate MSC migration through secretion of soluble factors such as EGF, VEGF-A, FGF, PDGF, SDF-1α/CXCL12, IL-8, IL-6, GMCSF, GCSF, Ang1, monocyte chemoattractant protein-1 (CCL2), haematopoietic growth factor, TGFβ-1 and urokinase-type plasminogen activator.

- The process of MSC mobilization to the tumour is thought to be regulated similarly to leukocyte migration through integrins and adhesion molecules.

- Molecules involved in leukocyte trafficking – such as tethering, rolling, adhesion and transmigration from the bloodstream to the tissue – are expressed on MSCs. These include integrins, selectins and chemokine receptors.

Tumor tropism of MSCs

- MSCs express a wide range of molecules, including growth factors, chemokines, adhesion molecules and toll-like receptors, on their surface.
- MSCs are known to functionally express chemokine receptors CCR1, CCR4, CCR7, CCR9, CCR10, CXCR4, CXCR5, CXCR6, CX3CR1 and c-met, which has been increasingly linked to tumour tropism.

- Although the tumor tropism of MSC is generally accepted, it depends on:

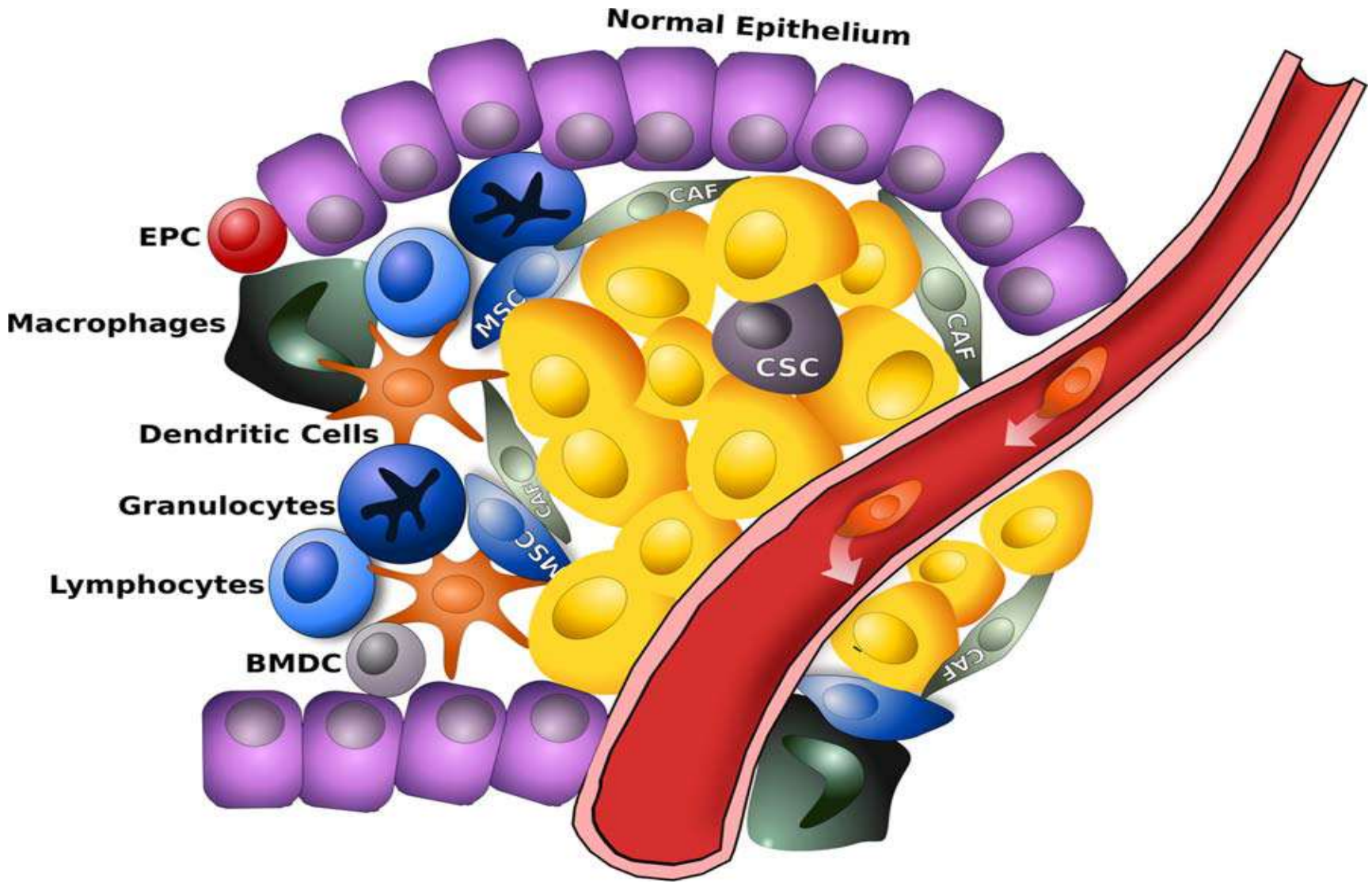
a. Tumor model
b. Different microenvironment created by different tumors
c. Degree of inflammation

The Role of MSCs in Neoplastic Microenvironment

- The bidirectional interplay between cancer cells and cells of stroma, including MSCs, endothelial, immune, and fibroblast-like stromal cells, plays a key role in tumor progression and metastasis and creates a complex microenvironment called tumor niche.

- In normal stroma, predominant cells are fibroblasts that secrete an extracellular matrix (ECM) providing a natural barrier against tumor progression. On the other hand, the ECM is able to support and promote tumor progression by modifications of the same ECM.

- In this context, both fibroblasts and myofibroblasts, denominated cancer-associated fibroblasts (CAFs) produce proteins such as collagen, fibronectin, a-smooth muscle actin, and others, creating alterations of ECM architecture. As a result, the cancer cells start to change their morphology becoming invasive and metastatic.



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The Role of MSCs in Neoplastic Microenvironment

- MSCs can be fundamental.

- MSCs can originate from tumor resident stroma progenitors, or can be recruited from other tissues as bone marrow by circulation.

- MSCs have the tendency to migrate into damaged tissues or organs, driven by chemotactic gradients of cytokines/chemokines released from same damaged tissues.

- Once arrived in injured sites, MSCs provide structural support and secrete factors for tissue repair. Therefore, this physiological behavior happens also for the tumor that can be considered as a “wounds that never heal”.

The Role of MSCs in Neoplastic Microenvironment

- Circulating MSCs from bone marrow, adipose tissue or MSCs derived from tumor stroma cells are able to differentiate in CAFs.

- In a model of inflammation-induced gastric cancer, MSCs generated CAFs that were recruited to tumor microenvironment in a process that was mediated by TGF-b and **SDF-1a**.

- In breast cancer, the axis SDF-1a/CXCR4 is crucial in the interaction between breast cancer cells and MSCs of bone marrow.

- Breast cancer cells are able to attract marrow derived MSCs and in turn, breast cancer cells preferentially metastasize to the bone marrow. In both cases, SDF-1a seems to be involved.

The Role of MSCs in Neoplastic Microenvironment

- Some studies describe two classes of polarized MSCs depending on expression of Toll like receptors (TLR):

i. TLR4 primed-MSCs are defined MSCs1 and are polarized into pro-inflammatory phenotype. They are able to inhibit tumor growth and metastasis.

ii. TLR3 primed MSCs are defined MSCs2 and have the classic immunosuppressive phenotype. They are capable to improve the tumor growth and favor metastasis formation.

- This classification depends on the cytokine profile expressed from specific MSCs and include over-expression of TGFb, and SMAD3–4.

The Role of MSCs in Neoplastic Microenvironment:
Exoxomes

- MSCs can also regulate the metabolism of cancer cells through secretion of exosomes.
- In a recent study, exosomes produced from the prostate cancers can inhibit the adipogenic differentiation of MSCs, favoring the differentiation of MSCs into myofibroblasts.
- In turn, the exosome differentiated MSCs stimulate angiogenesis and tumor growth.

Author	Exosome origin	Tumor model	Outcomes	Mechanisms
Li, Hongdan et al. [34]	Human bone marrow MSCs from patients undergoing hip-replacement surgery	Colon cancer cells (HCT-116, HT-29, and SW-480)	Increased the population of colon cancer stem cells	miR-142-3p in exosomes promoted the Notch signaling pathways by downregulating Numb
Zhang, Yanling et al. [35]	Human omental adipose-derived MSCs from cancer-free female donors	Human EOC cell lines (SKOV3, A2780, and HO-8910)	Promoted cancer progression	Affect proteomic profile of tumor cells via paracrine mechanism
Roccaro AM et al. [36]	Human bone marrow MSCs from normal or cancer patients	Multiple myeloma (MM) cells	MM BM-MSCs–derived exosomes promoted MM tumor growth, normal BM-MSC exosomes inhibited the growth of MM cells	Impact MM cell adhesion
Makiko Ono et al. [37]	Human bone marrow MSCs	BM2 cells	Slowed tumor growth	Exosomal transfer of miR-23b and its suppression of MARCKS
Reza AM et al. [38]	Human adipose MSCs	A2780 and SKOV-3 ovarian cancer cells	Inhibited proliferation of ovarian cancer cells	Upregulates proapoptotic molecules

Lin et al., 2019

The Role of MSCs in Neoplastic Microenvironment:
Macrophages

- Monocytes and macrophages can be recruited into tumors site altering the tumor microenvironment and accelerating tumor progression.
- Macrophages shift their phenotypes in response to various microenvironmental signals generated both from tumor and stromal cells.
- Macrophages can be subdivided into two categories: classic M1 and alternative M2 macrophages. The M1 macrophage is involved in the inflammatory response, pathogen clearance, and antitumor immunity; the M2 macrophage is involved in an anti-inflammatory response, wound healing, and has pro-tumorigenic properties.
- The tumor-associated macrophages (TAMs) closely resemble the M2-polarized macrophages and are critical modulators of the tumor microenvironment. TAM accumulation in tumors correlates with a poor clinical outcome and provide a favorable microenvironment to support tumor development and progression regulating tumor angiogenesis, invasion, metastasis, immunosuppression, and chemotherapeutic resistance.
- Together, MSCs and TAMs promote tumor growth. In fact, MSCs can promote tumor progression increasing recruitment of TAMs in tumor site via CCR2. Another chemokine produced by MSCs, able to recruit the TAM is CCL2. Thus, MSCs and TAMs can engage in a bidirectional interaction resulting in tumor promotion and progression.

Potential Role of MSCs in Tumourigenesis

- MSCs have also been implicated as tumour supportive when co-injected in the presence of a variety of tumour cell types, including breast, ovarian, melanoma, glioma and colon tumour cells. The majority of these studies, however, used an equal or even excess number of MSCs over tumour cells.
- MSCs were shown to integrate into the tumour stroma and were demonstrated to exert their effects at least partly through secretion of paracrine factors including CCL5, IL-6 and SDF-1α.
- There is also evidence that **MSCs may serve as precursors for carcinoma-associated fibroblasts and/or pericytes**, playing a potentially important role in tumour angiogenesis through differentiation and the release of proangiogenic factors.
- The immunosuppressive qualities of **MSCs may support tumour development and progression** through protection of cancer cells from immune surveillance.
- Conversely, co-injection of MSCs has also been shown to **result in tumour suppression** in a model of colon cancer, hepatoma and melanoma.

Discrepancy in Impacts of MSCs on Tumor Progression

- MSCs would be recruited into tumor sites, promoting tumor growth, and angiogenesis through diferentiating into cancer-associated myofibroblasts and secretion of proangiogenic cytokines (e.g., interleukin (IL)-6, vascular endothelial growth factor (VEGF), and transforming growth factor-β (TGF-β).
- The recruited MSCs also enhanced tumor metastasis via increasing lysyl oxidase.
- MSCs is attributed to their protection role for breast cancer cells from immune clearance through modulating regulatory T cells and inhibiting natural killer (NK) cells and cytotoxic T lymphocyte (CTL) functions.
- MSCs have been found to form a cancer stem cell niche in which tumor cells can preserve the potential to proliferate and sustain the malignant process.
- MSCs promote tumor angiogenesis through their potential to diferentiate into pericytes or endothelial-like cells as well as by their secretion of trophic factors and cytokines, pro-angiogenic factors, growth factors, and plasminogen activator .
- MSCs can afect tumor development and progression through miRNAs.

✓ Thus, MSCs promote tumor growth and metastasis through stimulation of angiogenesis, cancer stem cell niche maintenance, immune protection and miRNAs.

Author	MSC origin	Tumor model	MSC: tumor cell ratio	Outcomes	Mechanisms
Chaturvedi P et al. [17]	Human bone marrow-derived MSCs	Breast(MDA-MB-231, MDA-MB-43)	1:1 coinjection	Increased metastasis	activation of the hypoxia-inducible factors (HIFs)
Walter, M. et al. [21]	Human adipose stromal cells (ASCs)	Human breast cancer cell line MCF-7	1:1 coinjection	Increased migration and invasion	Secretion of IL-6
Tsai, Kuo–Shu et al. [22]	Human bone marrow-derived MSCs	Human colorectal cancer cell line HT-29	1:100 coinjection	Promoted tumor sphere formation and tumor initiation	IL-6 secreted by MSCs signaled through STAT3
Zhang, Ting et al. [23]	Human fetal bone marrow stem cells (hBM-MSCs)	4T1 mouse mammary tumor cell line	1:1 coinjection	Increased tumor growth	Neovascularization (secretion of macrophage inflammatory protein-2, vascular endothelial growth factor, transforming growth factor-beta and IL-6)
El-Haibi, Christelle P. et al. [24]	Human bone marrow-derived MSCs	MDA-MB-231 and MCF7/Ras breast cancer cells	1:1 coinjection	Enhanced metastasis	Increased de novo production of lysyl oxidase (LOX)
Patel, Shyam A. et al. [25]	Human bone marrow-derived MSCs	Highly aggressive MDA-MB-231 breast adenocarcinoma, low-invasive MCF-7 breast adenocarcinoma, T47D breast adenocarcinoma, P815 murine mastocytoma	1:1 (T47D and MSCs 2×10^5 /ml each) were added in 500 μl volumes to attain a 50:1 ratio of mononuclear fractions (PBMC)/MSC and PBMC/T47D	Protected breast cancer cells from immune clearance	Through Tregs, inhibited NK cell and CTL functions
Chandler, Emily M. et al. [26]	Human adipose-derived stem cells (ADSCs)	MCF-7 and MDA-MB-231	1:1 co-injection	Promoted tumorigenesis and angiogenesis	Bidirectional signaling; ADSCs differentiated into cancer-associated myofibroblasts
Gonzalez, Maria E. et al. [27]	Human breast cancer metastatic sites-derived MSCs	Breast cancer cell lines MDA-MB-231, MCF7, and MDA-MB-436	MSCs were orthotopically injected into the mammary fat pads (1×10^6 cells/mouse)	Loss of DDR2 in MSCs impaired their ability to promote DDR2 phosphorylation in BC cells, as well as BC cell alignment, migration, and metastasis	Reduced migration and metastasis

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Discrepancy in Impacts of MSCs on Tumor Progression

- The unmodifed MSCs have antitumor efects both in vitro and in different animal models of cancer, which is attributed to the factors secreted by MSCs that can suppress the proliferation of glioma, melanoma, hepatoma, and breast cancer cells.

- Human umbilical cord-derived MSCs (hUC-MSCs) have been shown to inhibit progression of breast cancer by inducing tumor cells death and suppressing angiogenesis.

- Human bone marrow-derived MSCs exhibit the potential to suppress the growth of breast cancer and inhibit lung metastasis by reducing their proliferative ability.

- MSCs have been shown to have anti-angiogenic efect both in vitro and in vivo.

✓ MSCs play critical roles in processes of tumor angiogenesis, tumor immune response, and metastasis.

Author	MSC origin	Tumor model	MSC: tumor cell ratio	Outcomes	Mechanisms
Ho, Ivy AW et al. [28]	Human bone marrow-derived MSCs	Primary human glioma cells	1:1 (coinjection)	Reduction in tumor volume and vascular density	Secretion of soluble factors inhibiting endothelial progenitor cells recruitment and impaired tumor angiogenesis
Leng, Liang et al. [29]	Human umbilical cord-derived MSCs	Human breast cancer cell line MDA-MB-231	1:1 (injection of MDA-MB-231 first, injection of MSCs 13 days later)	Antitumor effect	Inhibited tumor angiogenesis and induced cell apoptosis
Meleshina, Aleksandra V. et al. [30]	Human bone marrow-derived MSCs	MDA-MB-231 human breast adenocarcinoma cell line	1:1 (MDAMB-231-Turbo FP650 cells injection fist, injection of MSCs 10 days later)	Suppressed tumor growth and lung metastasis	Reduced proliferative activity of cancer cells
Dasari, Venkata Ramesh et al. [31]	Human umbilical cord blood-derived MSCs	Two high-grade human glioma cell lines (SNB19 and U251) and two xenograft cell lines (4910 and 5310)	1:4 (MSCs injection 7 days after tumor implantation)	Inhibited tumor growth	Upregulation of phosphatase and tensin homolog deleted on chromosome 10 (PTEN) in tumors induced cellular death through decreasing XIAP expression
Xie, Chan et al. [32]	Human bone marrow-derived MSCs (interferon beta (IFN-β) modified)	HCC cell lines HepG2 and Huh7	300:1 (MSC injection 3 days after HCC inoculation)	Inhibition of HCC proliferation	Inhibition of AKT/FOCO3a pathway
Wu, Ning et al. [33]	Human umbilical cords-derived MSCs (transfection of hepatocyte nuclear factor 4α (HNF4α)	Liver cancer cell lines HepG2 and SK-Hep-1	1:5 (MSC injection 24 h after tumor implantation)	Inhibited HCC proliferation and invasion	Downregulation of Wnt/β-catenin signaling in HCC cells
François, Sabine et al. [20]	MSCs from human or rat bone marrow	Colorectal cancer cell lines (HT29, HCT-116, LS513, and CC531)	N/A	Attenuation of tumor progression	Modulation of immune component

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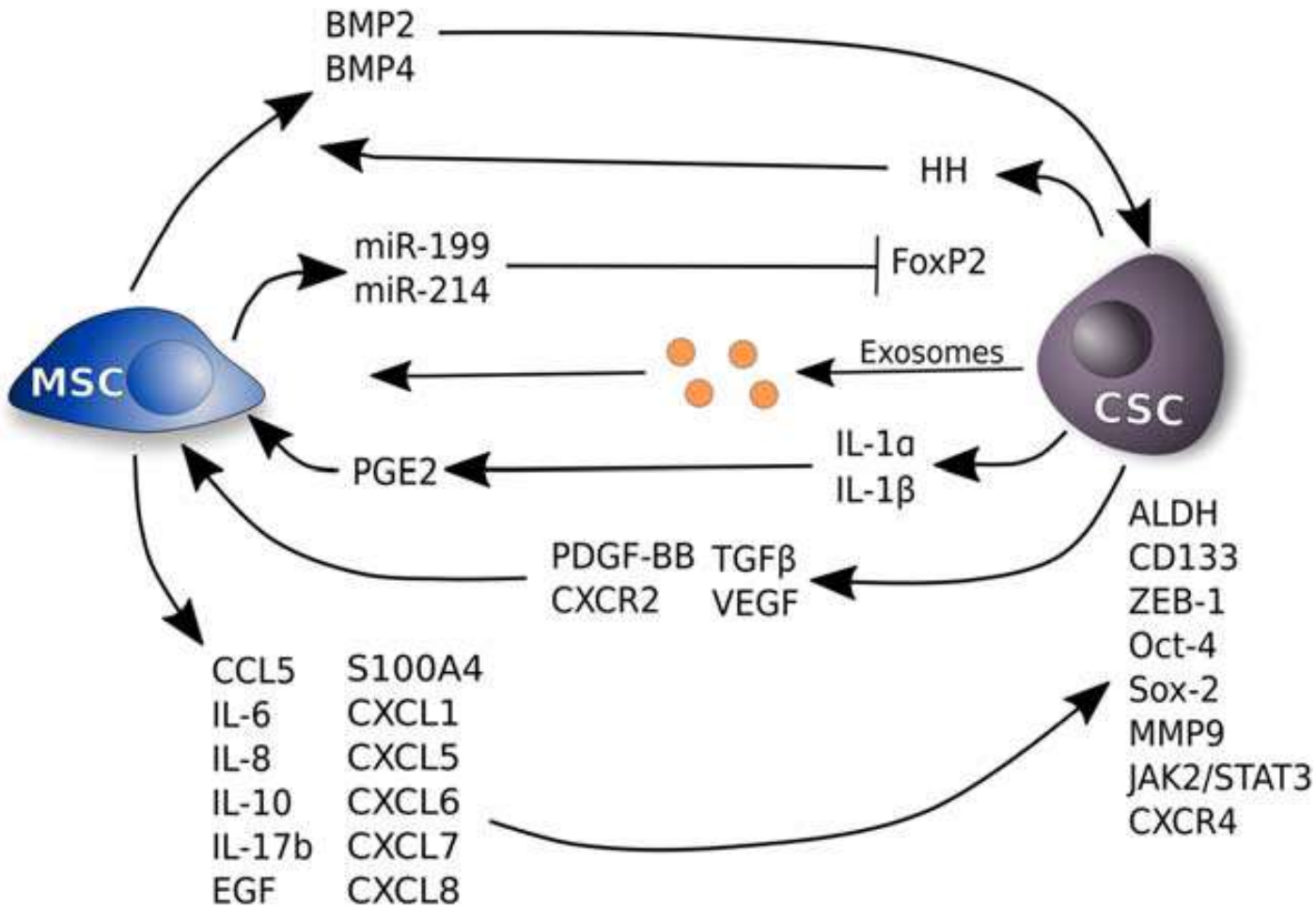
MSC, Cancer Cells and CSCs Interaction

- CSCs features are mainly represented by tumor-initiating capacity, metastatic potential, and drug resistance.

- Some studies have reported that MSCs can increase the CSCs population within a tumor.



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MSCs, Immune System and CSCs

- Human MSCs have been reported to partially express major histocompatibility complex class I and to lack the expression of HLA class II antigens, that may result in a non-immunogenic phenotype.
- Moreover, MSCs have been described as having immunosuppressive properties by modulating both T-cell and B-cell functions.
- These cells can be found around vascular areas of the bone marrow, which could have a negative effect on cytotoxic T cells.
- MSCs have been reported to exert an immune-suppressive effect. A probable mechanism may involve the demonstrated capacity of MSCs to migrate from bone marrow, adipose tissue and other sites, and to the tumor where they directly influence tumor microenvironment and tumor growth.
- Another mechanism may involve MSCs-mediated recruitment and maintenance of regulatory T cells (Tregs) resulting in cytotoxic T cells negative regulation, as it was demonstrated using bone marrow aspirate from healthy subjects. This expansion has been attributed to the secretion of TGF-b by MSCs.
- Besides favoring the expansion of Tregs, it has also been demonstrated that MSCs can induce a switch in favor of Th2-type CD41 T cells that increases expression levels of IL-10 and decreases the activity of NK cells.

MSCs, EMT and Metastasis

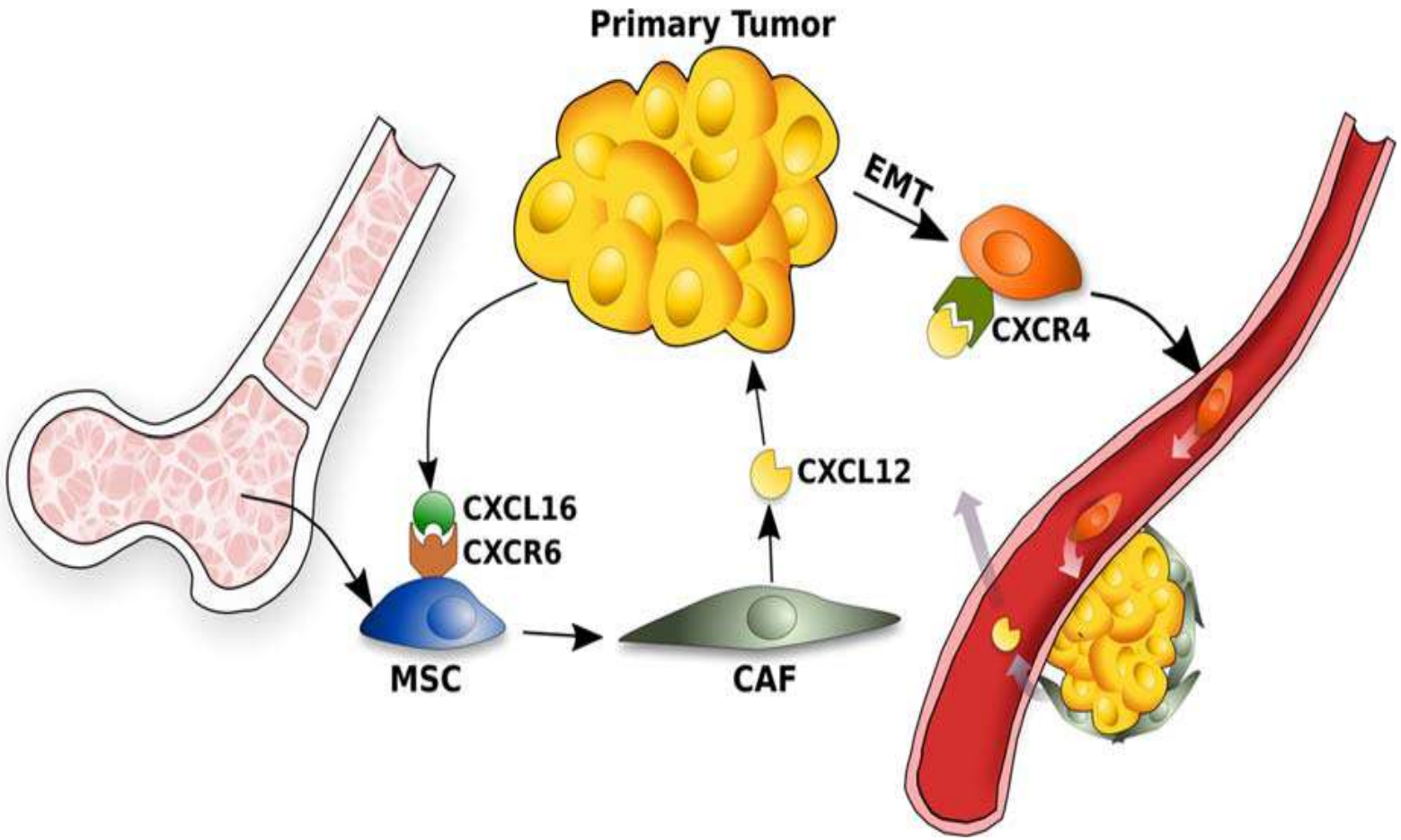
- EMT is a process through which cancer cells acquire an invasive phenotype that leads to metastasis. Some reports indicate a key role of MSCs in causing EMT. Human bone marrow-derived MSCs have been reported to promote EMT in pancreatic cancer cells through Notch signaling.

- During the metastatic process, MSCs can promote cancer progression by using homing and chemokines axis.

- Indeed, in a model of prostate cancer, it was demonstrated that prostate cancer cells secrete CXCL16 which recruits bone marrow-derived MSCs via the axis CXCL16/CXCR6.

- Subsequently MSCs differentiate into CAFs which, in turn, through the other axis CXCL12/CXCR4, induce EMT.

- By contrast, some reports have suggested an inverse role played by MSCs on the metastatic potential. For example, although human MSCs increased tumor growth, they also significantly downregulated TGF-b with effects on the invasive and metastatic potential and as demonstrated in a model of hepatocellular carcinoma.



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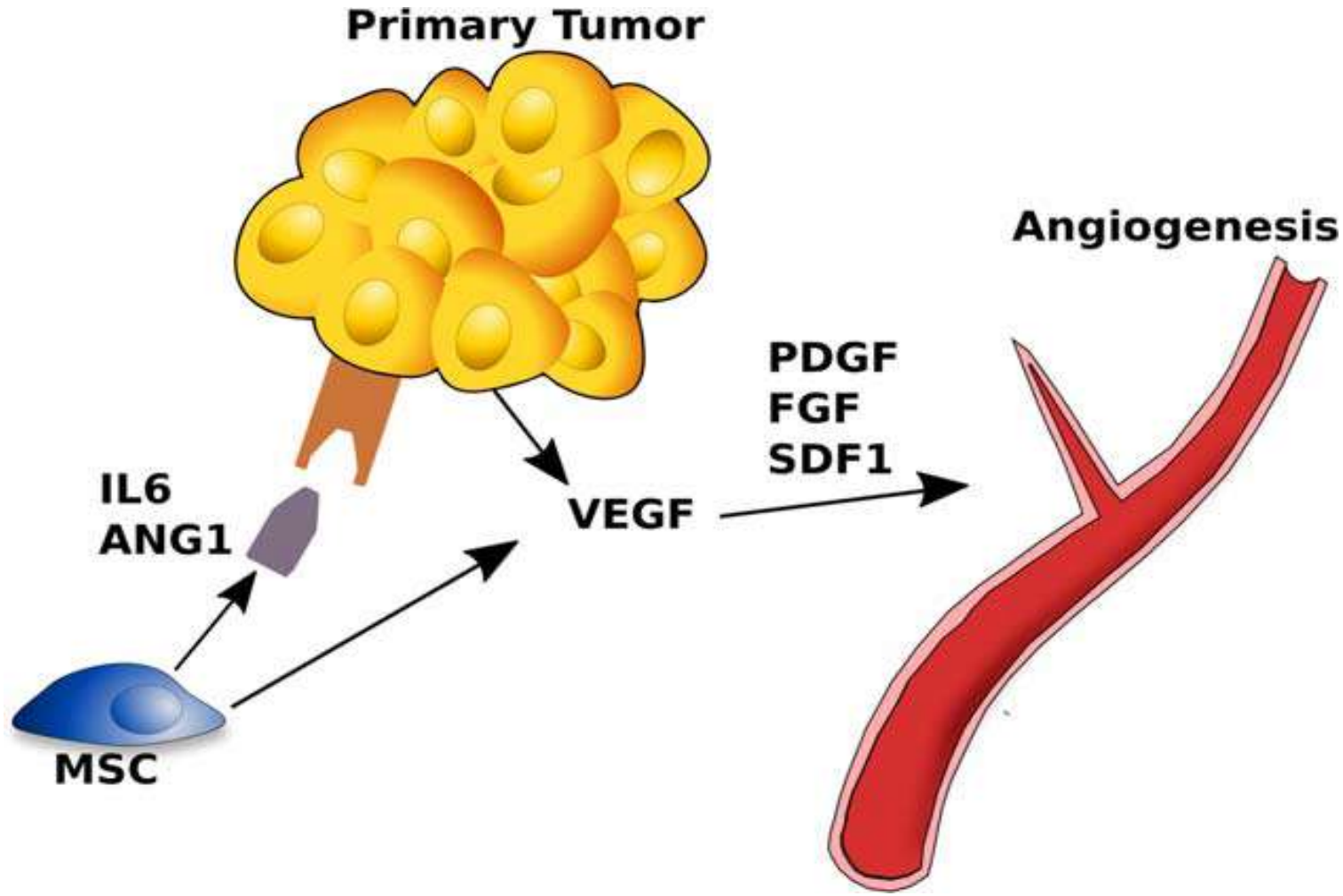
MSCs, Angiogenesis and CSCs

- Tumors create their own vascularization through different processes that are associated with angiogenesis, remodeling of preexisting vessels, recruitment, vascular mimicry (VM), and differentiation of bone marrow endothelial precursors.
- The vessel network is also a key component of the niche, where CSCs can play a role following radio- and chemo-therapies.
- MSCs that produce VEGF, Angiopoietin-1 (Ang-1) and other pro-angiogenic factors, could differentiate into pericytes and endothelial cells, which supports tumor vascularization and growth.
- In a colorectal cancer model, it has been shown that primary human MSCs secrete a series of pro-angiogenic factors, such as interleukin-6 (IL-6) and angiopoietin-1, inducing also cancer cells to produce endothelin-1 (ET-1), thereby promoting tumor angiogenesis.
- ET-1 activated Akt and ERK pathways in endothelial cells which led to the induction of tumor angiogenesis. In a breast cancer model, both endothelial cells and adipose-derived MSCs interplayed to give rise to pericytes and mature vessels.



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MSCs, Angiogenesis and CSCs

- Another process that deserves to be cited is the so called “vascular mimicry.” In this, cancer creates itself channels for fluid transport independent of typical modes of angiogenesis.

- Melanoma cells were able to increase the vasculogenic potential of MSCs by VM.

- In fact, MSCs derived from adipose tissues of C57BL/6 mice in cocultured with melanoma cells formed vascular-like network on Matrigel. MSCs alone was not able to form capillary like structures.

- This is the first direct evidence that melanoma cells instruct MSCs to participate in VM.

- Now, the concept of VM and its importance in interaction of MSCs and cancer cells is receiving improved attention in the field of angiogenesis especially for angiogenic therapies!

MSCs, Angiogenesis and CSCs

- However, there are opposite demonstrations of a negative role of MSCs on angiogenesis. It has been showed that murine MSCs could release reactive oxygen species which damage endothelial cells, and that MSCs could affect vessels formation in a melanoma model.

MSCs, CSCs and Multidrug Resistance

- The inefficacy of anticancer treatment may be ascribed to:

a. reduced drug uptake
b. increase in drug extrusion from the cancer cell
c. increase drug inactivation
d. decreased activation
e. decrease in the formation of drug activated complex
f. increase in repair of drug induced damage

CSCs are resistant to conventional therapies in many types of solid tumors.

MSCs, CSCs and Multidrug Resistance

- CSCs present some transmembrane transporters such as ABC transporters (ATP-binding cassette) family of molecules that actively pump the drug outside the cell.

- CSCs also possess some enzymes, like ALDH and glutathione transferase (GST), that are capable to metabolize and inactivate anticancer agents such as platinum salts and others.

- Several signaling pathways have been linked to the drug resistance of CSCs, among which Wnt, Notch, Hedgehog.

MSCs, CSCs and Multidrug Resistance

- A number of studies confirmed the capacity of MSCs to confer drug and radiation resistance to cancer cells.

- The methylation of the tumor suppressor genes promoters has been shown to transform MSCs into CSCs, that have tumor-initiating and drug resistance capacities in in vivo models .

- In general, MSCs can modulate the sensitivity of cancer cells to chemotherapeutic agents through the production of factors like polyunsaturated fatty acids, PDGF-C, hepatocyte growth factor, nitric oxide, and interleukin-17A.

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