



Review Article

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The Multifunction Role of Tumour-Associated Mesenchymal Stem Cells and their Interaction with Immune Cells

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Abstract

Mesenchymal Stem Cells (MSCs) are multipotent stromal cells that are present in the various parts of the body. MSCs tends to migrate towards inflamed regions and they are also seen to migrate to a tumour. The immunomodulatory properties of the MSCs were being studied for the past decade and it has been observed that these immunomodulatory properties of MSCs have been exploited by the cancer stoma. MSCs role in the tumour progression, stemness, malignancy and treatment resistance. In the breast cancer Tumour Microenvironment (TME) Immunomodulation by MSCs is mediated by a combination of cell contact-dependent mechanisms and soluble substances. Monocytes/macrophages, dendritic cells, T cells, B cells, and NK cells all show signs of MSCs' immunomodulatory capability. In a complicated interplay initiated by MSCs, anti-inflammatory monocytes/macrophages and regulatory T cells (Tregs) play a key role, as they unveil their full immunomodulatory potential. MSC-secreted cytokines are commonly blamed for the interaction between MSCs, monocytes, and Tregs. Here we review the current knowledge on cellular and molecular mechanisms involved in MSC-mediate-immunomodulation and focus on the role of MSCs played in the breast cancer progression and its TME. In this review we have also emphasised on the role of TAMs in TME and how they may aid in breast cancer progression, as well as the interaction between MSC and TAMs. A better understanding of the immunomodulatory interactions of MSCs with lymphocytes and TAMs and their interactions with the breast cancer matrix will allow the development of better therapeutic approaches to combat breast cancer.

Keywords: Breast cancer; Mesenchymal Stem Cells (MSC); Tumour microenvironment (TME); Tumour-associated macrophages (TAMS), Lymphocytes.

Abbreviations: MSC: Mesenchymal Stem Cells; TME: Tumour Microenvironment; TAMS: Tumour-associated Macrophages; ECM: Extracellular matrix; CAFs: Cancer-associated Fibroblasts; CFUs: Colony-forming unit Fibroblasts; Tregs: T regulatory cells; Bregs: Regulatory B cells; IFN- γ : Interferon-gamma; TNF- α : Tumour Necrosis Factor-alpha; IL: Interleukin; TGF- β : transforming growth factor β ; PGE2: Prostaglandin E2; CXCR : Chemokine Receptor; Blimp-1 - B lymphocyte-induced maturation protein-1; CCL: Chemokine motif ligand; EMT: Epithelial-mesenchymal transition.

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Introduction

Breast cancer is considered one of the common malignancies in women and an assorted disease at a molecular level. It is considered the 5th common cause of cancer death [1]. Early stages of breast cancers are treatable and have a higher survival rate, but coming to advanced stages of breast cancer the survival rate dips drastically. With the current treatment protocols, the early-stage breast cancers have almost 100% survival whereas advanced stage only has a 22% survival [2]. Therefore, there is a pressing need for understanding the Tumour microenvironment (TME) of breast cancer for the development of a better treatment protocol and management of the disease.

A cancer cell simply doesn't exist in isolation. It forms an environment where it dynamically interacts with other cells and non-cellular components to survive and grow and this is dubbed as TME. It is composed of blood and lymphatic vessels, fibroblasts, adipocytes etc acting as stromal cells lymphocytes, macrophages and other cells, extracellular matrix (ECM) and some secreted components [3]. In-depth studies have shown how crucial role TME plays in sustenance, metastatic progression, resistance and recurrence of the tumour [4,5]. There are studies which show Mesenchymal stem cells (MSCs) and macrophages as some of the predominant players of TME in breast cancer. The cross-talk between these two is being extensively studied and recognized as one of the pathways that can be used for therapeutic purposes in breast cancer [6,7].

Immune cells (and inflammation) have long been recognised as important in regulating and contributing to tumour growth. Both innate and adaptive immune components actively patrol the body for incipient tumour cells, and tumor-infiltrating lymphocytes with effector and memory activities have been extensively characterised inside primary tumours and their metastases. The existence of an immune infiltration is often linked with a positive prognosis; however, this is highly dependent on the tumour type, cell location, and level of activation.

Mesenchymal stem cells (MSCs) have attracted the attention of the scientific community and extensive work has been carried out for the past 3 decades for their immunomodulatory properties. MSCs like cells can be obtained from various tissues at all developmental stages (fetal, young, adult, and aged) but bone marrow is considered optimal source [8,9].

Immune cells may be seen throughout the TME, and they contain both innate and adaptive immune cell populations that interact with tumour tissue-associated MSC, with lymphocytes accounting for the bulk of tumor-infiltrating immune cells. Monocytes/macrophages, dendritic cells, T cells, B cells, and natural killer cells all show signs of MSCs' immunomodulatory capability. In a complicated interplay initiated by MSCs, anti-inflammatory monocytes/macrophages and regulatory T cells (Tregs) play a key role, as they unveil their full immunomodulatory potential.

Macrophages are specialised phagocytic cells in the innate immune system that play a variety of roles in homeostasis and immunological responses. Macrophages travel about continually as scavengers, removing dead cells, harmful bacteria, and other foreign substances. The activation of macrophages regulates and reacts to the immune system as a critical modulator and effec-

tor cell in the immunological response. The study of MSC-macrophage interactions in tissue homeostasis and injury healing has gotten a lot of interest in recent years. The control of MSCs on macrophages will be the focus of this review [10]. The immunomodulatory properties of MSCs were also attributed in breast cancer progression. The TME was able to subvert MSCs functions in favour of tumour progression [11].

In TME the immune system is one of the key players in the cross-talks that happen there. It was observed previously 5-40% of a solid tumour mass is populated by Tumour-Associated Macrophages (TAMS) that is associated with a poor prognosis [12]. Studies have reported that mammary tumours show a paracrine relation between TAMS and cancer cells. Several studies have shown an association between macrophage infiltration and angiogenesis in breast cancer. TAMS affect the progression of the tumour at every step like metastasis, including invasion, vascularization, intravasation, extravasation, establishing pre-metastatic niches [13,14].

TAMS help establish a microenvironment capable of facilitating mammary tumour immune evasion through the secretion of soluble factors [15,16]. The cues received from tumour and stromal cells push the macrophages towards M2 phenotype which are known for their anti-inflammatory, wound healing properties and pro-tumour properties. There are several studies which indicate the increase of TAMS in tumour micro-environment led to poor clinical outcome and they play a key role in the progression of angiogenesis, metastasis, immunosuppression and also leading to chemoresistance [17-19].

Looking at the roles played by MSCs and TAMS in the breast cancer hints that the crosstalk of MSCs and TAMS might be one of the major contributors for the sustenance and progression of TME and breast cancer.

In this review, we want to shed the light on the phenotypic and functional roles played by the MSCs and TAMS in breast cancer and the interactions between them aiding the progression of cancer and possible clinical immunotherapies that can be developed for the breast cancer targeting them.

Mesenchymal stem cells

The works of Friedenstein and his co-workers led to the discovery of mesenchymal stem cells (MSCs), nonhematopoietic stem cells. The term "mesenchymal stem cells" was proposed by Caplan in 1991 because of their ability to differentiate into more than one type of cells that form connective tissue in many organs [20]. In 2006, The International Society of Cellular Therapy (ISCT) specified few guidelines which were accepted by the scientific community and as per the guidelines the use of name multipotent mesenchymal stromal cells was recommended but mesenchymal stem cells (MSCs) remain in use. MSCs like cells can be obtained from various tissues, but MSCs derived from bone marrow and adipose tissue can create a larger number of CFU-F (colony forming units-fibroblast) colonies, which indirectly indicates a higher degree of their stemness [21]. The criteria to be met by cells to be called as MSCs is the growth of the cells in vitro to be adherent in nature and should express the cluster of differentiation (CD) markers like CD 73, CD 90, CD 105 and should lack the expression of CD45, CD34, CD14, CD11b, CD79a or CD19. These

cells also must possess the ability to differentiate into osteoblasts, adipocytes, and chondroblasts [22].

One of the key functions of the MSCs is their immunomodulatory properties. It was observed that the MSCs that were grown in vitro show the ability to interact and regulate the functions of key effector cells whose major involvement is seen in both innate and adaptive immunity.

In the past decade, compelling observations were suggesting certain patterns and pathways that have been constantly repeating which play a key role in MSC-mediated immunomodulation, operating through a balance in cell contact-dependent mechanisms and soluble factors [23-25]. It was also observed these immunomodulatory features were exploited by solid tumours like breast cancer. Let's take a look at these properties in brief.

The major cells of the adaptive arm of the immune system are T cells which are subdivided into different phenotypes like effector T cells, regulatory T cells (Tregs), and B cells. There are many studies which observed the modulatory effects of MSCs like suppression of T cell proliferation, inhibiting the differentiation of T cells into TH1 and TH17 subtypes while enhancing the proliferation of Tregs.

Immunomodulatory effects of MSCs on T cells

T cells are one of the important arsenals of the immune system against tumours. CD8+ T cells and CD4+ Th1 type T cells help in combating tumours using means like producing IFN- γ and cytotoxins. In breast cancer, having a high frequency of CD8+ T cells and CD4+ Th1 type T cells leads to a favourable prognosis [26,27]. However, when higher levels of Th2 and Tregs are seen in breast cancer the prognosis is poor and difficult in treating [28,29].

T cells are categorised into various subsets and each plays a fundamental role in breast cancer progression. The complete picture of their role is still unclear [42]. It has been observed that in the early stages of breast cancer there is an accumulation of Th17 and Treg cells, with the progression of the tumour the level of Th17 gradually decreases and the Treg cells increase [43].

It has been shown that MSCs can suppress the T cell proliferation which is induced by the mitogens in vitro. MSCs cause a shift in T-cell polarization from pro-inflammatory Th1 to anti-inflammatory Th2 cells and also in their secreting cytokine profiles [30-32]. It has also been observed that MSCs were able to regulate the production of proinflammatory cytokines like IFN- γ , IL-17 and TNF- α by TH1 and TH17 cells. It has also been reported that MSCs were able to boost the production of anti-inflammatory cytokines, like IL-4 (TH2). These reports the ability of MSCs to favour the polarization of TH1/TH17 towards TH2 response. MSCs when stimulated by IFN- γ and TNF- α /IL-1 shows their immunosuppressive potential. MSCs also release indoleamine 2,3-dioxygenase (IDO), which is enhanced when they are stimulated by IFN- γ . Following tryptophan deprivation, allogeneic T cell responses are inhibited, IL-4 secretion in Th2 cells is stimulated, and IFN- γ production in Th1 cells is decreased.

The MSCs show their suppressive potential on Th1 cytokine production through PGE2 dependent manner and TH17 through the up regulation of PD-1 expression and up regulation of IL-10 production. It has also been seen that CCL2 dependent suppres-

sion is also used by MSCs for their suppressive functions [33-36]. As we have seen MSCs have immunomodulatory properties which might be playing a key role here and it has been shown that decrease of TH17 and increase in Tregs leads to poor prognosis and tough to treat [28,29]. There are also studies showing MSCs promoting the proliferation of regulatory T cells (Tregs). It has been observed that when MSCs were cocultured with peripheral blood mononuclear cells (PBMCs) they promote the CD4+ T cells differentiation into Tregs through cell-cell contact-dependent manner. So, the Tregs induced by the MSC are not expanded from the existing Tregs, but from the induction of conventional T cells [37-39].

There are studies which show increase intertumoral infiltration of CD4+ T cells in breast cancer but it did not bode well with prognosis of the disease [40]. There is also the involvement of soluble factors like IL-10, TGF- β , IL-6 and PGE2 in this conversion. There are studies showing that MSCs may directly limit the proliferation of all reactive CD4+ and CD8+ T cells in the absence of other immune cells, a process mediated in part by MSC-derived galectin-1. MSCs can decrease T cell activation and promote permanent T cell hypo-responsiveness and death via secreting PD-L1 [41].

Immunomodulatory effects of MSCs on B cells

Amongst the tumour infiltrating lymphocytes B cells make a major portion in breast cancer [44]. The diverse functions of B cells like antigen presentation, cytokine production and interaction with other immune cells enables them to modulate the immune system pushing the immunity from pro-tumours to anti-tumours. But there are recent reports which show presences of Bregs in breast cancer which doesn't give out a favourable prognosis [45].

MSCs suppressive potential has also been seen on B cells. MSCs were able to inhibit the characteristics of B cells like activation, differentiation, antibody production, proliferation and chemotaxis. MSCs arrest the proliferation of B cells in the G0/G1 phase in a paracrine manner. MSCs were able to directly interact with B cells and can downregulate the plasma-blast formation and promote the development and induction of regulatory B cells (Bregs) [46]. The Bregs were able to push CD4+ T cells into developing into Tregs through the production of IL-10. The promotion of IL-10 producing Bregs by MSCs is dependent on cell-to-cell contact mechanism but not through secretory soluble factor mechanism. So, for this to happen MSCs should be in a metabolically active state [47,48].

PD-1 and PDL1 interaction also play a key role in the MSCs suppressive nature on B cells. It has also been observed that MSCs were able to suppress the conversion of B cells into Plasma cells by down regulating a master transcription regulator Blimp-1 (B lymphocyte-induced maturation protein-1) which is required for the B cell terminal differentiation [49,50]. MSCs were also able to inhibit the chemotaxis of B-cells by down regulating the expression of receptors like CXCR4, CXCR5, CXCL12 [46].

MSCs interact directly with B cells, reducing plasma blast development and promoting the induction of regulatory B cells (Bregs). Bregs promote immunological tolerance by its immunosuppressive characteristics. Bregs that produce IL-10 have been demonstrated to convert Foxp3+ Tregs from effector CD4+ T cells.

MSCs' stimulatory influence on B-reg formation and IL-10 production appears to be dependent on direct cell-cell contact or at least close proximity to the relevant cells, rather than soluble substances. However, it has been demonstrated that MSCs' stimulatory impact on B-reg creation and inhibitory influence on T cell proliferation are both dependent on active cell metabolism. MSC-secreted IL1-RA suppresses B cell development through a cytokine-triggered mechanism. MSCs reduce B cell growth in the presence of T cells, which might be owing to IFN- γ secreted by T cells, as IFN-pre-treated MSCs can also inhibit B cell proliferation.

Infiltrations of Bregs in the solid tumours like breast cancer has been identified. There are various studies which suggest accumulation of Bregs in the TME might one of the major processes through which B cells regulate various arms of immunity in the TME [45]. As the evidence suggests, in the conversion of recruited B cells into regulatory phenotype MSCs might be playing a major role which play role in breast cancer progression [48].

Immunomodulatory effects of MSCs on NK cells

The Natural killer cells are one of the major lymphocyte population that plays a key role in the innate arm of immunity. They exert their cytolytic activity either through Perforin involved pathway or through a caspase-dependent pathway. In the presence of IL-2 and IL-15, they get activated and MSCs were able to inhibit the proliferation driven by this mechanism. MSCs were also seen in interfering with the NK cells ability to produce pro-inflammatory cytokines and cytotoxic molecules like granzyme and perforin. MSCs also down regulate the expression of activating receptors like NKp30, NKp44 and NKg2D on NK cells [51-53].

Tumor-associated MSCs (T-MSCs) have been found to be one of the main cells responsible for immunosuppression in the context of malignancies by producing PGE2. T-MSCs have also been shown to have a strong inhibitory effect on NK cells by down regulating the expression of NKG2D, DNAM-1, and NKG2A on NK cells via direct cell-to-cell contact. Galland et al. (2017) conducted a comparative research on T-MSCs directly obtained from lung squamous cell carcinoma tissues, MSCs from neighboring normal tissues, and BM-MSCs to investigate the suppressive capacities of these MSCs of various origins. It was discovered that, unlike BM-MSCs, which had a substantial immunosuppressive impact on NK cells, those obtained from normal tissues had a less effect. While their findings showed that T-MSCs from tumor-bearing tissues close to normal sites from which normal MSCs were obtained had more potent suppressive features than BM-MSCs, they also found that T-MSCs from tumor-bearing tissues close to normal sites had more potent suppressive features than BM-MSCs. The inflammatory milieu of the tumour site, which was caused by tumour cells, stromal cells, and even immune cells, drove the MSCs of the tumour tissues to reclaim powerful BM-MSC-like suppressive capabilities, it was then pointed out [110].

There were recent observations which show the involvement of NK cells in HER2+ breast cancer [54]. It has been reported the MSCs were able to exert their immunosuppressive effects on NK cells by secretion of indoleamine-2,3-dioxygenase (IDO) and prostaglandin E2 (PGE2) [65]. MSCs are also known to secrete a soluble isoform of MSC class II molecule HLA-G5 which tends to interact with the inhibitory receptors (CD94-NKG2A, KIR2DL4 and ILT-2) on NK cells and inhibit the NK cells cytotoxicity functions. These NK

cell ligands play an important role in the immune editing of the tumour and associated with the immune escape of breast cancer. It was observed that upon exposure to IFN- γ MSCs becomes more resilient towards NK cells by up regulating the MHC class I expression which inhibits the NK cells function. IFN- γ exposure also increases the expression of inhibitory proteins like COX2 and IDO in MHCs [55]. In conditions like breast cancer, the cytotoxic potential of tumour infiltrating NK cells was substantially impaired compared to the peripheral NK cells which are correlated to the decrease in expression of activating receptors (NKG2D, etc) and increase in expression of inhibitory receptors (NKG2A, etc) leading to the survival of cancer [56].

Immunomodulatory effects of MSCs on dendritic cells

Dendritic Cells (DC) act as a bridge between the innate and adaptive immune systems, through presenting the antigen to T-cells and playing a key role in regulating their activation and functions, they also tend to affect B and NK cells by interacting with them directly [57,58]. Depending upon their activation levels and subsets DCs can either be immune-activating or regulating. In their immature state DCs express no co-stimulatory molecules and like mature DCs they possess the ability to recognize, process and present the antigen to T-cells, but in the absence of co-stimulatory receptors on DCs during interaction push these T-cells towards anergy or apoptosis. Several studies are showing the MSCs inhibiting the maturation and function of DCs, further inhibiting the activation and proliferation of T cells. DCs can directly inhibit the maturation of monocytes and the precursor cells of DC.

In a tumour microenvironment, MSC have been found to induce monocyte polarisation toward an anti-inflammatory/immune-regulatory (type 2) phenotype and to hinder dendritic cell development towards the type 1 phenotype (DCs) [115]. MSCs also prevent DCs from migrating and maturing. DCs are less capable of supporting antigen-specific CD4+ T cell proliferation and displaying an MHC class II-peptide complex in the presence of MSCs. When mature type 1 DCs are co-cultured with MSCs, they produce much less TNF- α , whereas anti-inflammatory mature type 2 DCs secrete significantly more IL-10 [111,116]. Sca-1+CD117L in bone marrow-derived MSCs have also been proven in mice to create regulatory DCs with immune regulation functions from hematopoietic stem cells [117].

Another question about DC-MSC interactions is whether MSC can interfere not only with DC formation from precursors, but also with later phases of differentiation, such as the transition from immature to mature DC. Several research organisations looked into this topic and came up with conflicting conclusions. MSC were shown to moderately decrease LPS-induced monocyte-derived DC maturation in some circumstances. When compared to control mDC, the resultant cells had a lesser capacity to promote allogeneic T cell proliferation in MLR, as well as lower levels of IL-12 production and IFN- γ induction.

There are reports in breast cancer patients, DCs have a substantially lower level of expression of HLA-DR and also show lower expression of MHC-II [59]. The MSCs were able to inhibit DCs at several levels through various regulatory molecules like IL-6, PGE2, Jagged-2, TSG-6, M-CSF [60-62]. Interaction of DCs with MSCs generates low endocytic capacity, low immunogenicity, and strong immunoregulatory function DCs. They also have significantly re-

duced expression of ligands, CD11c, CD80, CD86, and CD40 while increased expression of CD11b [63] MSCs also secreted Galectin-1 (Gal-1) which upregulated the expression of Gal-1 in DCs which in turn helped in the formation of tolerance immunophenotype on DCs, by the regulation of the MAPK signalling pathway [64]. DC's seems to be pushed towards tolerogenic subtype in the tumour environment and MSCs were reported to have a significant role in it in breast cancer [65]. Such DCs could lead to the induction of Th2 and Tregs and thereby result in the suppression of pro-inflammatory T cell activation.

Immunomodulatory effects of MSCs on monocytes

MSC have been demonstrated to enhance monocyte/macrophage polarisation toward an anti-inflammatory/immune-regulatory (type 2) phenotype while directly inhibiting development into the type 1 phenotype [111,112]. Anti-inflammatory monocytes secrete a lot of IL-10 and have lower levels of IL-12p70, TNF- α , and IL-17 expression, thanks to MSC-produced IL-6 and hepatocyte growth factor (HGF) [113]. In a positive-feedback loop, monocyte-derived IL-10 limits monocyte development into DCs and pushes monocytes toward an anti-inflammatory, IL-10-secreting subtype. MCS-primed monocytes express significant amounts of MHC class II, CD45R, and CD11b, in addition to IL-10, and appear to be able to limit T-cell activity in the absence of FoxP3+ Tregs. CCL-18 generated by monocytes and transforming growth factor beta 1 (TGF β -1) secreted by monocytes are both involved in monocyte-induced Treg development [114].

Immunomodulatory effects of MSCs on macrophages

Macrophages (M ϕ) are one of the innate immune system cells that are known for their phagocytic activity and immune properties. They are generally categorized into two phenotypes, the M1 macrophages a pro-inflammatory phenotype and M2 macrophages an anti-inflammatory or regulatory phenotype. They are categorized depending on their secretory profile and the surface receptor expression. Studies were showing the MSCs working in symbiosis with macrophages to maintain tissue homeostasis.

M ϕ have a lengthy life lifetime in diverse tissues, with some surviving for months to years. Despite the fact that M ϕ have the ability to proliferate in tissues, they seldom divide and are mostly replaced by monocyte migration in the blood. M ϕ can be separated into two types based on distinct activation methods: conventionally activated M ϕ (M1) and alternatively activated M ϕ (M2). Surface receptor expression, cytokine and chemokine production, effector functions, and so on differ between the two kinds.

M1-type cells can be activated by cytokines such as interferon gamma (IFN- γ), lipopolysaccharide (LPS), granulocyte-macrophage colony stimulating factor (GM-CSF), or tumour necrosis factor (TNF), which results in increased self-antigen presentation, complement-mediated phagocytic activity, proinflammatory factor release (IL-1, TNF-, IL-12, IL-6, IL (CXCL9, CXCL10, etc.) M1 cells can stimulate the removal of non-self-components in vivo and play an essential role in tumour prevention by releasing these inflammatory mediators. M1 cells also act as effector cells in the Th1-mediated immune response, increasing inflammation and killing intracellular pathogens.

M2a, M2b, and M2c cells are subtypes of M2-type cells. M2a is triggered by IL-13 or IL-4, whereas M2b is triggered by Toll-like

receptor (TLR) ligands and IL-10. M2a and M2b cells primarily regulate the immune system by encouraging the Th2-mediated immune response. M2c cells' major job is to prevent the occurrence of the immune response, which is crucial in the process of tissue remodeling.

In an inflammatory environment when the monocytes enter, they respond to the local stimuli and either differentiate into M1 macrophages secreting cytokines like IFN- γ and TNF- α and add to the inflammation or they might develop into M2 macrophages and secrete IL-10 and TGF- β trying to douse the inflammation. The transition of monocytes to M1 or M2 is influenced by many factors and the threshold of type of signal they receive also influence them in this transition.

The macrophages, which are influenced by MSCs likely to have an increased proliferative and migratory potential [66]. Macrophages influenced by MSCs have a great potential in impairing T-cell response and inducing Tregs. MSCs under the action of IFN- γ and TNF- α in an inflammatory setup gains a greater ability to influence the shift of macrophages from M1 to M2 by expressing higher concentrations of COX2 and IDO and also induces Tregs at a greater capacity and inhibit effector T cell response [67,68]. Certain pathways had been identified through which MSCs influence macrophage functions. It was observed that MSCs released factors like TNF-stimulated gene 6 (TSG6) which effected the TLR2-nuclear factor- κ B (NF- κ B) signalling which hindered the activation of peripheral macrophages [69].

In breast cancer tumour micro-environment setup, MSCs were observed to release a plethora of cytokines like CCL-2, CCL-7 and CCL-12 due to which there is increased recruitment of CCR2 expressing monocytes and macrophages which aid in the tumour progression [70] (Figure 1).

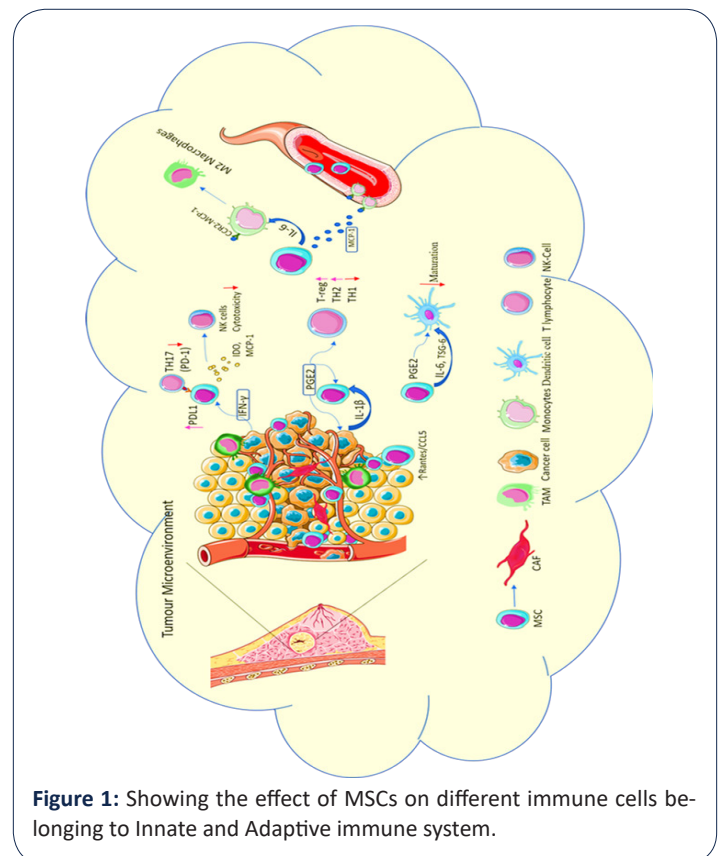


Figure 1: Showing the effect of MSCs on different immune cells belonging to Innate and Adaptive immune system.

The MSCs are found in different parts of the body which were able to migrate all over the body as well as into the tumour [71]. Due to the chronic inflammatory conditions, neovascularization and infiltration of immune cells tumours are considered “wound which doesn’t heal” it enables TME to recruit MSCs as well. Though bone marrow is considered a major source the MSCs also spring from the adipose tissue surrounding it [72].

The MSCs in the TME undergo differentiation into CAFs and express FAP (Fibroblast activation protein) and FSP (Fibroblast specific protein) whereas the FAP expression is generally not detectable in healthy tissues [73-75].

In a tumour microenvironment, the immune system is one of the key players in the cross-talks that happen there. It was observed previously 5-40% of a solid tumour mass is populated by tumour-associated macrophages (TAMs) that is associated with a poor prognosis [12,14]. TAMs are the macrophages that were recruited and educated in the tumour microenvironment. They are exposed to regulatory cytokines like IL-10 and TGF- β making them M2 like phenotype [76,77]. Thanks to their M2 like phenotype TAMs were observed to carry out immunosuppressive function rather than immune effector [14].

The dominant phenotype of TAMs in breast cancer is M2 phenotype which is known for their tumour promoting [78]. TAMs are known to promote tumour growth, invasion, angiogenesis [79,80]. Studies have reported that mammary tumours show a paracrine relation between TAMs and cancer cells. Monocyte colony-stimulating factor receptor (M-CSFR, also known as CSF-1R or cFMS) is expressed by TAMs, which binds to monocyte colony-stimulating factor (M-CSF, also known as CSF-1) secreted by cancer cells. Likewise, Epidermal Growth Factor (EGF) secreted by TAMs and activate the EGF receptor (EGFR) on the cancer cells. This facilitates the co-migration of the two cell types, which enhances the motility and subsequent invasion into surrounding healthy tissue [15,81].

Several studies have shown an association between macrophage infiltration and angiogenesis in breast cancer. TAMs affect the progression of the tumour at every step like metastasis, including invasion, vascularization, intravasation, extravasation, establishing pre-metastatic niches [13,16,82,83]. TAMs expressing the macrophage Colony-Stimulating Factor (CSF1) and its receptor (CSF1R) correlated to poor prognosis in breast cancer [84].

Macrophages present in mammary tumours undergo a profound reduction of MHC class II expression mediated by tumour-derived Migration Inhibitory Factor (MIF), inhibiting subsequent antigen presentation and adaptive immune induction. Owing to their abundance within mammary tumours, the loss of tumoricidal function by macrophages represents a crucial breach in immunosurveillance required for breast cancer development and progression [85] (Figure 2).

MSCs role in Cancer and crosstalk with TAMs

In recent decades in-depth studies have been conducted on the relation between MSCs and Cancer. But there isn’t any conclusive answer to this question. There are studies showing cancer progression and metastasis promoting actions by MSCs [86,87]. There are also studies showing the suppressive effect of MSCs on the proliferation of Tumour. Studies had shown recruitment

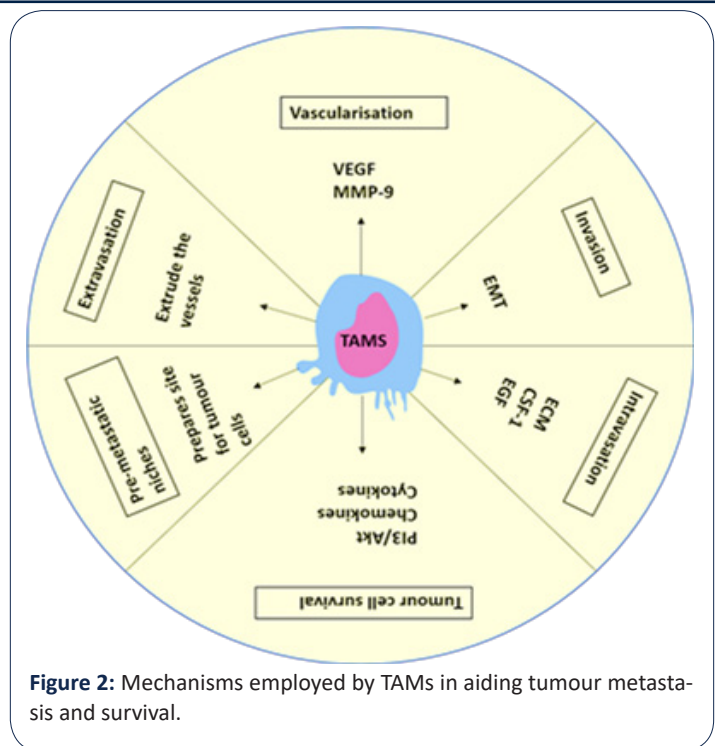


Figure 2: Mechanisms employed by TAMs in aiding tumour metastasis and survival.

of MSCs to the tumour sites which promoted the growth of the tumour. The transformation of MSCs into cancer-associated myofibroblasts was also observed which secrete the angiogenic cytokines like IL-6, VEGF and TGF- β [88-90]. These recruited MSCs promote metastasis by lysyl oxidase up regulation. The immunomodulatory properties of MSCs were also attributed in breast cancer progression. The MSCs modulation of Tregs and down regulation of NK cells and Cytotoxic T Lymphocytes (CTL) functions helped in breast cancer progression [91,92]. There is the recruitment of MSCs to the tumour microenvironment where they differentiate into Cancer-Associated Fibroblasts (CAFs) which promotes the tumour progression.

MSCs secrete an array of molecules depending on the environmental cues it gets. In tumour microenvironment also they secrete several molecules which play an important role in the tumour’s fate. In ovarian cancer, MSCs were found to secrete (Bone morphogenetic Proteins) BMP2 and 4 which increases the number of cancer stem cells (CSCs) [93]. In-turn this CSCs activate and up regulate the Hedgehog pathway. In breast cancer, MSCs were found to induce and up regulate the expression of mir-199 and mir214 leading to the down regulation of FoxP2 promoting survivability if CSCs and progression of metastasis [94]. The cancer cells release IL-1 α and IL-1 β which induces the expression of PGE2, IL-6 and IL-8 by MSCs leading to the production of CXCL1 and CXCL8 promoting the stemness characteristics in the tumour microenvironment.

MSCs were able to propagate the breast cancer cells positive for Aldehyde Dehydrogenase (ALDH) through the production of CXCR2 which induces the expression of Sox2 and Oct4 [95,96]. MSCs also secrete IL-6 which up regulates the CD133 expression through the JAK2-STAT3 pathway in CSCs. Studies are showing when MSCs were cocultured with breast cancer cells, promoted the expression of CXCR2 ligands including CXCL 1,5,6,7 and 8 which supported the developments of CSCs. There is also an increase in breast cancer CSCs when MSCs secrete cytokines like IL-10 IL-17b and proteins like EGF. MSCs were also seen regulating

the metabolism of CSCs through exosome production in breast cancer. CCL5 secreted by MSCs promotes the growth of breast cancer and its invasive properties [97,98]. In the progression of tumour macrophages are one of the immune cells that demands due attention. The macrophages and monocytes are recruited to the tumour microenvironment which alters and accelerate the tumour progression. The cues received from tumour and stromal cells push the macrophages towards M2 phenotype which are known for their anti-inflammatory, wound healing properties and pro-tumour properties. These M2- polarized macrophages closely resemble the Tumour-associated Macrophages (TAMs) that are important for the tumour microenvironment. There are several studies which indicate the increase of TAMs in tumour micro-environment led to poor clinical outcome and they play a key role in the progression of angiogenesis, metastasis, immunosuppression and also leading to chemoresistance [17-19].

The metastasis initiation requires the invasion which is triggered by Epithelial-Mesenchymal Transition (EMT) pathway. This is a process in which the epithelial cell loses its cell-to-cell adhesion and cell polarity and gain migratory and invasive properties. The MSCs present in the tumour microenvironment may stimulate the EMT pathway of tumour cells. Studies are showing breast cancer cells, when co-cultured with human bone marrow, derived MSCs showed increased EMT markers (N-cadherin, vimentin, Twist and Snail) and decrease of E-cadherin [99,100].

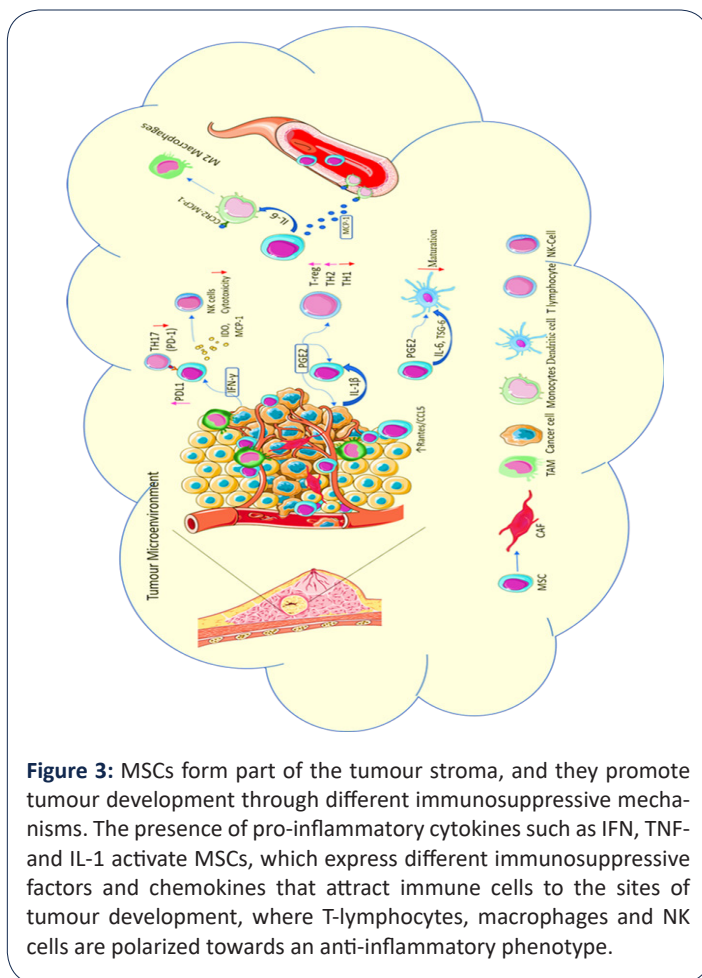


Figure 3: MSCs form part of the tumour stroma, and they promote tumour development through different immunosuppressive mechanisms. The presence of pro-inflammatory cytokines such as IFN, TNF- and IL-1 activate MSCs, which express different immunosuppressive factors and chemokines that attract immune cells to the sites of tumour development, where T-lymphocytes, macrophages and NK cells are polarized towards an anti-inflammatory phenotype.

In breast cancer it was observed, adipose-derived MSCs induce an upregulation of EMT related genes. The method of action through which MSCs exerts its effect on the tumour is not yet fully elucidated but there are studies which reported when the MSCs were cocultured along with breast cancer cells, they promoted the elongation, directional migration and traction of cancer cells. All this was possible through MSCs secreted TGF- β , focal adhesion kinases, matrix metalloproteases and migratory proteins [101] (Figure 3).

In tumour microenvironment the MSCs secrete a varied series of growth factors, cytokines and chemokines which are known to influence the breast cancer TME and help in tumour progression, migration and angiogenesis [102].

The impact of cytokines on metastasis, angiogenesis and tumour progression. The type and levels of cytokines vary amongst various stages of breast cancer i.e., from early to metastatic [103]. The breast cancer TME exhibit an inflamed cytokine profile leading to a poor clinical outcome [104,105]. Upon the progression of breast cancer progression to advance stages the expression of TGF- β is increased which helps in the tumour stemness, immune suppression and treatment resistance [106]. Reports are showing the production of TGF- β by MSCs which play one of the key roles in the metastasis of breast cancer [101] and also enhances the EMT progression [91]. In the breast cancer upon the interaction of IFN- γ and TNF- α , MSCs tends to produce TGF- β promoting EMT, migration and invasion of breast cancer [107]. Breast cancer cells also secrete high levels of IL-6 which also attracts and activates the MSC under the hypoxia conditions [108]. The MSCs also tend to secrete IL-6 in breast cancer TME in hypoxia condition and promotes tumour immune evasion and polarization of macrophages to M2 phenotype [107]. IL-6 by MSCs also stimulates STAT-3 phosphorylation and promotes breast cancer cell progression and migration the tumour growth and metastasis is also promoted by MSCs by secreting enzyme matrix metalloproteinase16 (MMP16) [99]. MSCs were able to modulate the stemness of the breast cancer cells through cytokine production like IL-6 and chemokine ligands -7 (CXCL-7) [109].

Conclusion

The role of MSCs has been implicated in several stages of the tumour progression. Due to the secretion factor secreted by the cancer cells recruit the MSCs to cancer microenvironment. In the TME the MSCs are tend to differentiate into CAFs which help in the tumour progression. In the breast cancer environment, MSCs promote the EMT of cancer cells which play a key role in the cancer progression. MSCs also promote breast cancer metastasis

MSCs through their immunomodulatory properties had been seen to affect the immune cells that invade the breast cancer TME and promote the cancer immune escape, treatment resistance. Through paracrine and direct contact action the MSCs were able to promote the stemness and angiogenesis. Even though studies are showing the MSCs anti-tumour affect the overall net effect seems to be tumour progression

In this review we have discussed MSCs and the mechanisms through which they exert their immunomodulatory effect on both adaptive and innate immunity in the cancer microenvironment, emphasising both cells to cell interaction and paracrine effect. We

have also discussed the role of TAMs in TME and how they help in breast cancer progression and the cross-talk between MSCs and TAMs. Having a better understanding of immunomodulatory interaction MSCs with lymphocytes and TAMs and their cross-talk with breast cancer stroma will enable in designing a better treatment modality for combating breast cancer.

Declarations

Conflict of interest: The authors declare that no competing financial interest.

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