

## Review Article

# Mesenchymal Stromal Cells: from Bone Marrow to Neoplastic Disorders

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## Abstract

Mesenchymal stromal cells (MSCs) represent a small and heterogeneous subpopulation of mesenchymal stem cells that possesses multilineage differentiation potential. These cells are mainly present in bone marrow, but also in other tissues, and represent a valuable resource for their ability to differentiate into different cell lines and for many therapeutic approaches. MSCs are able to differentiate into cells of mesodermal origin such as adipocytes, chondrocytes, osteoblasts or fibroblasts and *in vitro* also into cells of non-mesodermal lineages. In bone marrow, they establish the microenvironment for the growth and differentiation of the hematopoietic stem cells (HSCs) resulting crucial for HSC maintenance and haematopoiesis. Nevertheless, the proliferation and/or the survival rate of MSCs may contribute to the onset of different types of bone sarcomas, such as Osteosarcoma, Chondrosarcoma and Giant Cell Tumor of Bone that represent the result of neoplastic degeneration of their corresponding committed mesenchymal precursors, probably as a consequence of the alteration of different or common biochemical pathways.

**Keywords:** Mesenchymal stromal cells; Bone marrow; Neoplastic disorders; Stem cells; FGFRs

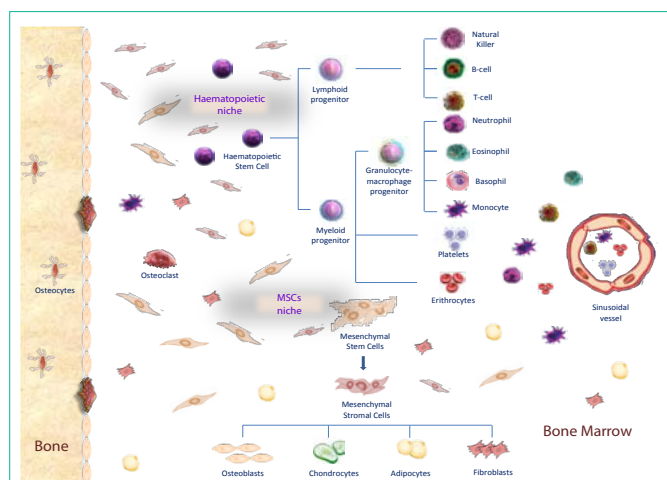
## Bone Marrow and Stem Cell Niches

Bone plays an essential role in the structure and movement of the body, and consists of cells (osteoclasts and osteoblasts) at different developmental stages, collagen fibrils, and mineral deposits such as calcium and phosphate. The bone cavity is filled with soft bone marrow that is the primary postnatal site of several stem cells including those of haematopoietic and mesenchymal lineages [1-5]. The stem cell niche represents the microenvironment created by supporting cells and their signals, in which stem cells reside and undergo self-renewal and differentiation [6-7]. The ability of adult stem cells to self-renew and differentiate is a critical point for tissue homeostasis: the depletion of this population occurs as a consequence of boosted self-renewal rate. On the other hand, the uncontrolled expansion of stem cells population, could promote tumorigenesis. The quiescence of stem cells within the niche is essential, about 70% of which are in the G<sub>0</sub> phase of the cell cycle. Particularly, it has been shown that approximately 30% of the quiescent Haematopoietic Stem Cell (HSC) divide every 145–193 days (protecting them from DNA damage by limiting the number of their cellular divisions), while a more active subpopulation divides every 28–36 days [8]. One mechanism that ensures the balance between self-renewal and differentiation processes is the control of asymmetric/symmetric stem cell division. In asymmetric division the stem cells divide into 2 daughter cells: one daughter cell remains in the niche as a stem cell and the other leaves the niche to produce a committed cell population. In symmetric division stem cells divide into 2 identical daughter cells that remain both in the niche as stem cells. The last decade has witnessed an increasing interest in stem cells niches, even if the number of niches in bone marrow, their cellular composition as well as their interactions are yet to be clearly determined [6,9-15]. The biochemical pathways promoting Mesenchymal Stem Cells (MSCs)

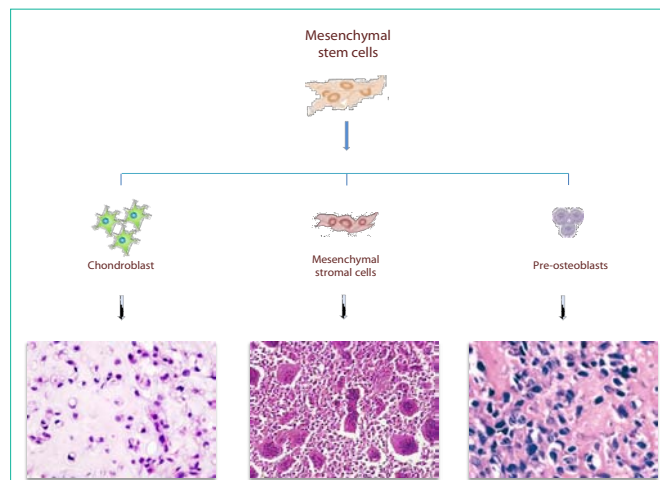
commitment are activated by micro-environmental conditions that include hormones (PTH, vitamin D3 and estrogen), growth factors (BMPs, TGFβs, IGF), and mechanical stimuli. In addition, there is an increasing interest in the role of non-coding RNAs (miRNAs) as well as of epigenetic mechanisms regulating the differentiation fate of MSC [16-18].

## Mesenchymal Stromal Cells Differentiation

Haematopoietic stem cells mainly reside within the bone marrow, which is the primary site of HSC maintenance and haematopoiesis and also contains many other different non-haematopoietic cell types (Figure 1). Stromal and differentiated cells (chondrocytes, osteoblasts, fibroblasts, adipocytes) compose this microenvironment, normally referred as stroma [19,20]. Particularly, mesenchymal stromal cells that are committed toward osteoblast lineage express bone sialoprotein, osteonectin, osteopontin, osterix and Runx2 (runt-related transcription factor 2) [21]. Many different biochemical pathways drive this osteoblast differentiation program including Fibroblast Growth Factors (FGFs) and WNTs signaling [22,23]. Specifically, FGF receptor ligands are involved in proliferation and osteoblast differentiation of mesenchymal precursors and therefore in bone deposition, through the interaction with four type of fibroblast growth factor receptors (FGFRs) [24,25]. FGFR3 and FGFR4 are involved in the differentiation of chondrocytes. On the other hand FGFR1 is involved in osteoblast proliferation while FGFR2 promotes osteoblast differentiation of mesenchymal stem cells promoting Runx2 expression and inhibiting TWIST1 [26,27]. Also Wnt signalling is involved in MSCs proliferation and in the regulation of the osteogenic differentiation [28]. Canonical Wnt signaling can be activated by the interaction of several secreted Wnt ligands with frizzled receptors and with the co-receptor Lipoprotein



**Figure 1:** Bone marrow niches and stem cells differentiation. In *Haematopoietic niche*, MSCs contribute to the creation of microenvironment that promote haematopoietic stem cells differentiation in myeloid and lymphoid progenitors, that differentiate in several blood components such as lymphocytes, granulocytes, platelets and erythrocytes. In *Mesenchymal Stem Cell niche*, MSCs differentiate in mesenchymal stromal cells and then in osteoblasts, chondrocytes, adipocytes and fibroblasts in response to different transcription factors.



**Figure 2:** Mesenchymal origin of different bone sarcomas. Mesenchymal origin of different bone sarcomas deriving from different committed stromal-related cells: Osteosarcoma, derives from uncontrolled proliferation of MSCs committed to osteoblastic lineage; Giant Cell Tumor of bone is caused by neoplastic proliferation of immature mesenchymal stromal cells; Chondrosarcoma, derives from uncontrolled proliferation of MSCs committed to chondroblastic lineage.

Receptor Related Protein 5/6 (LRP5/6). Wnt activation, involves different effectors such as  $\beta$ -catenin, JNK and calcium-channels regulators. Cytoplasmatic  $\beta$ -catenin accumulation and its nuclear translocation promote the activation of several oncogenes (e.g., c-Myc) and of different metalloproteinases, promoting extracellular matrix (ECM) degradation and cellular invasion and migration [29]. Wnt3a ligand has a modulatory function in chondrogenesis through bone morphogenetic protein (BMP)-2 expression; Wnt7a enhances chondrogenesis through various TGF $\beta$ 1-MAPK signaling pathways; and Wnt1 inhibits chondrogenesis promoting TWIST1 up-regulation [30].

### Mesenchymal Stromal Cells in Solid Tumor

Recent studies showed that MSCs are involved in tumorigenesis, being able to integrate into solid tumors [31,32]. Marrow stroma formation is essential for tumor growth and requires the interaction between malignant tumor cells and non-malignant stromal cells [31,32]. Mesenchymal stromal cells represent the neoplastic component of different bone sarcomas: Osteosarcoma, Chondrosarcoma and Giant Cell Tumor of Bone Figure 2).

#### Osteosarcoma

Osteosarcoma (OS) is the most common primary solid malignant tumor of the bone that occurs in the metaphyseal regions of long bones, mainly in young patients [33]. Almost constantly intramedullary, it may rarely originate at the bone surface. OS shows high tendency and resistance to conventional chemotherapeutic treatment and the majority of secondary recurrences are due to pulmonary metastasis [34]. This bone sarcoma is a consequence of genetic and epigenetic alterations in mesenchymal progenitor cells committed toward osteoblastic lineage that produce osteoid and/or immature bone resulting in sarcomatous degeneration [35]. Several studies hypothesized that this tumor could derive from less mature precursors of osteoblast cells because these cells are able to differentiate

in chondroblastic, fibroblastic and osteoblastic components [36]. Germline mutations in retinoblastoma (Rb) and in Tumor suppressor p53 (TP53) genes are associated with OS development. Other genes are probably involved, but the high rate of genetic instability that characterizes this tumor complicates the identification of the causative gene. Interestingly, OS patients show aberrant activation of the Wnt signaling due to an accumulation of  $\beta$ -catenin in the cytoplasm or in the nucleus [37]. Therefore, Wnt signaling hyper activation enhances the expression of c-myc oncogene, responsible for neoplastic proliferation of OS cells, and of several MMPs (MMP-9 and MMP-14), responsible for OS metastatic invasion and associated with poor disease survival [38]. The mesenchymal nature of OS cells is also supported by GLI2 over-expression in OS patients, a transcription factor whose over-expression enhances mesenchymal stem cells proliferation and accelerates cell cycle progression [39-41].

#### Giant cell tumor of bone

Giant Cell Tumor of Bone is an aggressive osteolytic bone neoplasm composed of three major cell types: mesenchymal stromal cells, mononuclear (CD68 positive) histiocytic cells and multinucleated osteoclast-like giant cells [42]. Although this tumor mainly arises in the epiphyses of long bones of the appendicular skeleton, it can also occur in other areas [43]. Histologically, GCT lesion is made up of several multinucleated giant cells that are uniformly distributed among mononuclear spindle-like stromal cells and monocytes. Particularly, mononuclear histiocytic cells (MNHC) and multinucleated giant cells are considered to belong to the monocytic-histiocytic system, because both cell lines express CD68 antigen. It is widely accepted that MNHC and MNGC are secondarily recruited and do not constitute the neoplastic cell population. In fact, the neoplastic component of the tumor is represented by spindle-like stromal cells, which are able to proliferate in vitro and to form tumors in mice [44]. A recent study definitively demonstrated that MSCs represent the neoplastic component of GCT because only stromal cell compartment (CD51-/CD61-/CD14-) show somatic

mutations (G34W or G34L) in *H3F3A* gene, which is mutated in 92% of conventional GCT patients [45]. Conversely, *H3F3A* mutations are not detected in giant cell osteoclasts-like (CD51+/CD61+), that represent the osteolytic component of GCT. Giant cells derive from hematopoietic precursors and their formation is directed by the stromal cells that produce several chemokines, including stromal cell-derived factor-1 (SDF-1) and monocyte chemoattractant protein-1 (MCP-1), that recruits monocytes to the tumor site [46,47]. Besides, stromal cells also produce macrophage colony-stimulating factor (M-CSF) that is responsible for monocytes proliferation and differentiation [48]. M-CSF also induces RANK expression on monocytes [49]. The mechanism by which *H3F3A* mutations drive GCT onset is unknown but it can be hypothesized that histone 3.3 mutations directly lead to the alteration of expression of FGF and/or Wnt signaling. Recent studies demonstrated that GCT patients showed high expression of FGFR2IIIc and TWIST1, two osteogenic markers that regulate MSCs terminal osteoblast differentiation [50-52]. Indeed, the mechanism through which FGFR2-IIIc contributes to MSCs uncontrolled proliferation remains unclear; it could be speculated that high levels of FGFR2IIIc should promote MSCs osteoblastic differentiation through Runx2 expression. TWIST1 high levels inhibit Runx2 expression and cause the maintenance of mesenchymal stromal cells in immature state. The high recurrence GCT rate upon surgical removal may result from residual stromal cells that are capable of re-forming the tumor that expresses Stro-1 was reported to have stem-like properties [53]. Again, the neoplastic role of the mesenchymal component is supported by the identification that MMP-2 and MMP-9 are expressed in the stromal cells [21]. Microarray analysis confirmed MMP-9 high expression in whole GCT tumor samples and in stromal cells [54,55]. In conclusion, GCT is bone sarcoma whose genetic lesion has recently been identified arising as a result of the uncontrolled proliferation of MSCs.

### Chondrosarcoma

Conventional chondrosarcomas represent about 90% of all chondrosarcomas and are divided according to their location in primary and secondary. The majority is primary and arises in intramedullary cavity of bone and is classified into three grades of malignancy (from I to III) [56,57]. Less differentiated chondrosarcomas cells, show more similarity with MSCs, while more differentiated chondrosarcomas share similarities with fully differentiated chondrocytes [58]. Histological and immunohistochemical analyses reveal that chondrosarcoma consists of cells that are in a different differentiation state [59]. The neoplastic component of chondrosarcoma is unknown and no evidence of neoplastic degeneration of adult chondrocytes has been reported. However, the mesenchymal nature seems evident especially for grade III chondrosarcomas, because in its muco-myxoid matrix the cells at the periphery of the lobules may become spindle-shaped, resembling a less differentiated phenotype [60]. Therefore, although the mesenchymal nature of this extremely heterogeneous tumor remains dubious, there is a growing body of evidence identifying it as an additional tumor that results from uncontrolled proliferation of mesenchymal cells that are committed toward chondrocyte line.

### Mesenchymal Stromal Cells in Cancer Therapy and Other Clinical Conditions

MSCs have generated a great interest in oncology for their

ability to repair which makes them particularly suitable in cell-based therapies [61]. Moreover, because of their remarkable capacity to be recruited from bone marrow into the blood circulation and then into damaged sites, MSCs could be used as vehicles for anti-cancer drugs [62,63]. After intravenous infusion, MSCs accumulate in liver cancer-derived structures as well as in tumor stroma in breast cancer and osteosarcoma, while in hematological malignancies MSCs autologous transplantation improves hematopoietic stem cells engraftment in bone marrow [63-66]. Tumor-tropic migratory properties of MSCs derive from stimuli produced by the tumor tissue (chemokines) and from their intrinsic properties (chemokine receptors) [67]. For this reason, MSCs have also been used as vehicles to efficiently deliver oncolytic viruses into tumors and metastatic sites in models of breast carcinoma, ovarian cancer and glioma [68-71]. The tumor-suppressive effects of MSCs are due to the down-regulation, through Wnt inhibitors, of Wnt signaling target genes that are involved in anti-apoptosis, cell proliferation and cell cycle regulation [72,73]. This effect could also derive from their ability to inhibit NF- $\kappa$ B pathway in cancer cells and to produce tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), inhibiting different type of tumor growth [74,75]. Therefore, the mechanisms of MSCs-based cancer therapy are not yet completely clear and further studies are required to ensure the quality and bio-safety of MSCs. MSCs properties also represent an important bio-resource for novel cell and gene-based therapeutic strategy of several non-tumoral conditions. Their capacity to regenerate mesenchymal tissues strongly supports their use in regenerative medicine to replace or repair damaged tissues of mesenchymal origin [76]. Moreover, for their ability to trans-differentiate into cell lineages belonging to not-mesoderm embryonic layers (e.g. neurons, liver, kidney and spleen), MSCs represent a useful tool for the treatment of several medical conditions including stroke, spinal cord injuries, acute kidney failure or act as *multidrug dispenser* to favour tissue regeneration [77,78]. The driving force of MSCs use derives from their role in immune response modulation as they show low inherent immunogenicity, which allows their use for both autologous and allogeneic cell therapies. The non-immunogenic property of MSCs is due to the absence of the expression of class II MHC molecules on their surface that render them able to inhibit T cells activation [79,80].

### Conclusion

Studies focused on mesenchymal stromal cells are important to accumulate evidence and information to define their nature for therapeutic approaches. Moreover, this type of information seems crucial to prevent neoplastic degeneration of their committed cell or at least to design better therapeutic approaches for affected patients.

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