

## The Role of Mesenchymal Bone Marrow-derived Cells in Tumor Formation

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By definition, mesenchymal stem cells (MSCs) are highly proliferative precursor cells that differentiate into multiple cell types, including adipocytes, osteoblasts, and chondrocytes. In conditions of increased cell turnover, such as wound healing, tissue remodeling, or bone growth, MSCs are recruited from the bone marrow and contribute to the formation of new tissues (1). Similarly, cellular regeneration is increased in the formation of the tumor stromal compartment, which includes inflammatory cells, blood vessels, and fibroblasts that may be generated by bone marrow-derived MSCs (2). A recent study showed that perivascular progenitor cells in tumors are recruited from the bone marrow and express phenotypic markers, including Sca-1, CD11b, PDGFR- $\beta$ , and c-kit, which have been observed in culture-expanded MSCs and MSC progeny (3). Other studies have described the formation of a bone marrow-enriched 'niche' which stimulates the migration and engraftment of leukemic cells in the mouse skull (4) and Lewis lung carcinoma cells in the lungs (5). We hypothesize that mesenchymal bone marrow-derived cells modulate solid tumor growth, thus serving as a potential target for cancer therapeutics. A thorough characterization of the phenotype of mesenchymal bone marrow-derived cells that engraft in the tumor stroma is warranted and will permit the development of new strategies to (i) specifically target these cells to prevent/delay tumor progression; or (ii) use MSCs as carriers to deliver therapeutic payloads to tumors.

First, we investigated the phenotype of MSCs isolated from the bone marrow of C57BL/6 and FVB mice by adherence to plastic in culture by immunostaining and flow cytometry. Briefly, culture-expanded MSCs (passages 3-5) were trypsinized for 10-15 minutes at 37 C and collected by centrifugation. Cells were separated into 100  $\mu$ l aliquots and labeled with 2% Sca-1-PE, CD45-PerCP, CD31-FITC, CD31-APC, CD11b-PE, and/or CD117-APC (c-kit). Intriguingly, the bone marrow cells isolated from C57BL/6 mice possessed a more myeloid/monocytic phenotype (CD45<sup>bright</sup> CD11b<sup>+</sup>c-kit<sup>+</sup>), whereas the bone marrow cells harvested from FVB mice displayed a more typical mesenchymal phenotype (Sca-1<sup>bright</sup> CD45<sup>-</sup> CD31<sup>-</sup>). Furthermore, we found that primary bone marrow cells from FVB mice expressing GFP under the elongation factor 1-alpha (EF1 $\alpha$ , an ubiquitously expressed gene) promoter, expressed extremely low levels of GFP. Nevertheless, MSCs isolated and expanded from EF1 $\alpha$ -GFP mouse bone marrow homogenously expressed high levels of GFP. Therefore, we used this model for the *in vivo* studies described below.

Second, to determine whether mesenchymal bone marrow-derived cells spontaneously engraft into tumors *in vivo*, P0008 mammary carcinoma tumors were implanted in the mammary fat pad of FVB mice. These mice were previously given lethal irradiation (10 Gy) and rescued by a restorative bone marrow transplant from EF1 $\alpha$ -GFP mice. When the tumors measured 1 cm in diameter, the blood (collected from the vena cava), bone marrow (from the tibia and femur), and tumor cell suspensions (obtained after digestion of tumor tissue with 2 mg/ml collagenase II) were collected and the expression of GFP, CD45, and Sca-1 were analyzed by flow cytometry. As expected, GFP-expression in blood and bone marrow was very low; however, tumors expressed detectable levels of

GFP, indicating that a substantial fraction of tumor-infiltrating mesenchymal cells were derived from the bone marrow.

In summary, using quantitative multi-color flow cytometry we investigated the phenotype of bone marrow-derived mesenchymal stem cells cultured *ex vivo* and compared that to the phenotype of bone marrow-derived cells spontaneously infiltrating into tumors. We found that mesenchymal stem cells contribute significantly to the formation of tumors. We believe that a better understanding of the role of mesenchymal bone marrow-derived cell homing, engraftment, and proliferation in tumor formation will assist in the development of MSC-based therapeutics.

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