Mesenchymal Stem Cells: Flip Side of the Coin

Pravin J. Mishra,[1] Prasun J. Mishra,[4] John W. Glod,[2,3] and Debabrata Banerjee[1,2]

[1]Departments of Medicine, [2]Pharmacology, and [3]Pediatric Oncology, The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey; and [4]Laboratory of Cancer Biology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland

Abstract

Tumor-associated fibroblasts or carcinoma-associated fibroblasts (CAF) play an important role in the growth of epithelial solid tumors. Although the cell type of origin of CAFs has not been conclusively established, it has been shown that they may be bone marrow derived. One side of the mesenchymal stem cell (MSC) coin is the well-accepted therapeutic potential of these cells for regenerative and immunomodulatory purposes. The ominous dark side is revealed by the recent work demonstrating that hMSCs may be a source of CAFs. In this review, we discuss the role of stromal cells in the tumor microenvironment and suggest that by exploring the *in vitro*/*in vivo* interplay between different cell types within the tumor milieu, strategies for improved tumor therapy can be developed. [Cancer Res 2009;69(4):1255–8]

Introduction

Two major types of stem cells are present in the bone marrow, the hematopoietic stem cell and the nonhematopoietic or mesenchymal stem cells (MSC). Under appropriate conditions, MSCs can give rise to cells of muscle, bone, fat, and cartilage lineage (1). The ability of MSCs to self-renew and differentiate makes them a promising avenue for clinical applications in regenerative medicine (2, 3). MSCs localize to sites of hematopoiesis, sites of inflammation, and sites of injury as well as to solid tumors (4–6). The ability of MSCs to migrate to tumor sites has encouraged investigation into the possibility of using these cells as a gene delivery mechanism (7, 8). Naive MSCs have been shown to inhibit tumor growth, prompting the use of these cells as tumor inhibitory cells *in vivo* (9). Furthermore, immunosuppressive effects of MSCs have been used for therapy of graft-versus-host disease. It has also been shown that coinjection of MSCs favors growth of B16 melanomas in allogeneic mice due to the immunosuppressive effect of MSCs (10, 11). The wide range of therapeutic applications of MSCs representing one side of the coin is covered in recent reviews (3, 12, 13). The focus of this article is to present the flip side of the coin. In this review, we argue that bone marrow–derived MSCs can be a source of carcinoma-associated fibroblasts (CAF), which may contribute to tumor growth in several ways. We further propose that by studying these cells *in vitro*, we can begin to reconstitute the tumor microenvironment. This strategy may facilitate development of important therapeutic interventions to control tumor growth that are based on interfering with the interaction between diverse cellular components of solid tumors (Fig. 1).

Bone Marrow–Derived MSCs as Source of Tumor/Carcinoma-Associated Fibroblasts or Myofibroblasts

Accumulating evidence suggests that tumor-associated fibroblasts or CAFs play an important role in the growth of epithelial solid tumors. It has long been known that a significant fraction of the stroma in some breast cancers consists of fibroblasts (14). More recent studies show that CAFs from breast cancer specimens promote tumor cell growth compared with fibroblasts obtained from non-neoplastic locations. In addition to tumor growth, the tumor stroma has also been implicated in other important processes such as angiogenesis and metastasis. Orimo and colleagues (15) defined several important characteristics of breast CAFs including promotion of breast carcinoma cell growth, promotion of angiogenesis, and expression of myofibroblast traits. Expression of the chemokine stromal-derived factor 1 (SDF-1) has also been shown to be important in the interaction between tumor cells and stromal fibroblasts (15).

Although the cell type of origin of myofibroblasts has not been conclusively established, it has been shown that they are bone marrow derived (16). In a recent study, we have shown that human bone marrow–derived MSCs (hMSC) exposed to tumor-conditioned medium (TCM) over a prolonged period of time assume a CAF-like phenotype (17). More importantly, these cells exhibit functional properties of CAFs including sustained expression of SDF-1 and the ability to promote tumor cell growth both *in vitro* and in an *in vivo* coimplantation model. These CAF-like MSCs also express myofibroblast markers including α-smooth muscle actin (α-SMA) and fibroblast surface protein. Gene expression profiling revealed similarities between TCM-exposed hMSCs and CAFs. This suggests that hMSCs are a source of CAFs and can be used in modeling tumor-stroma interactions (17, 18).

Further evidence for a bone marrow source of tumor-associated fibroblasts comes from studies using a gastric cancer mouse model (Gan mice) in which prostaglandin E2 (PG E2) and Wnt signaling were simultaneously activated in the gastric mucosa. As both PGE2 and Wnt pathways play a role in human gastric tumorigenesis, the Gan mouse model may recapitulate important aspects of the molecular etiology of human gastric cancer. Bone marrow transplantation experiments indicated that subsets of gastric myofibroblasts were derived from bone marrow (19).

Alterations in Tumor-Associated Stromal Cells

Although the importance of cross-talk between cancer cells and other components of the microenvironment has been increasingly recognized, the question of whether the stromal cells themselves harbor cancer-promoting mutations is just beginning to be addressed. For example, Patocs and colleagues (20) hypothesized that mutational inactivation of the tumor-suppressor gene TP53 and

Requests for reprints: Debabrata Banerjee, Department of Medicine and Pharmacology, The Cancer Institute of New Jersey, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, 195 Little Albany Street, New Brunswick, NJ 08903. Phone: 732-235-6458; Fax: 732-235 8181; E-mail: banerjed@umdnj.edu.

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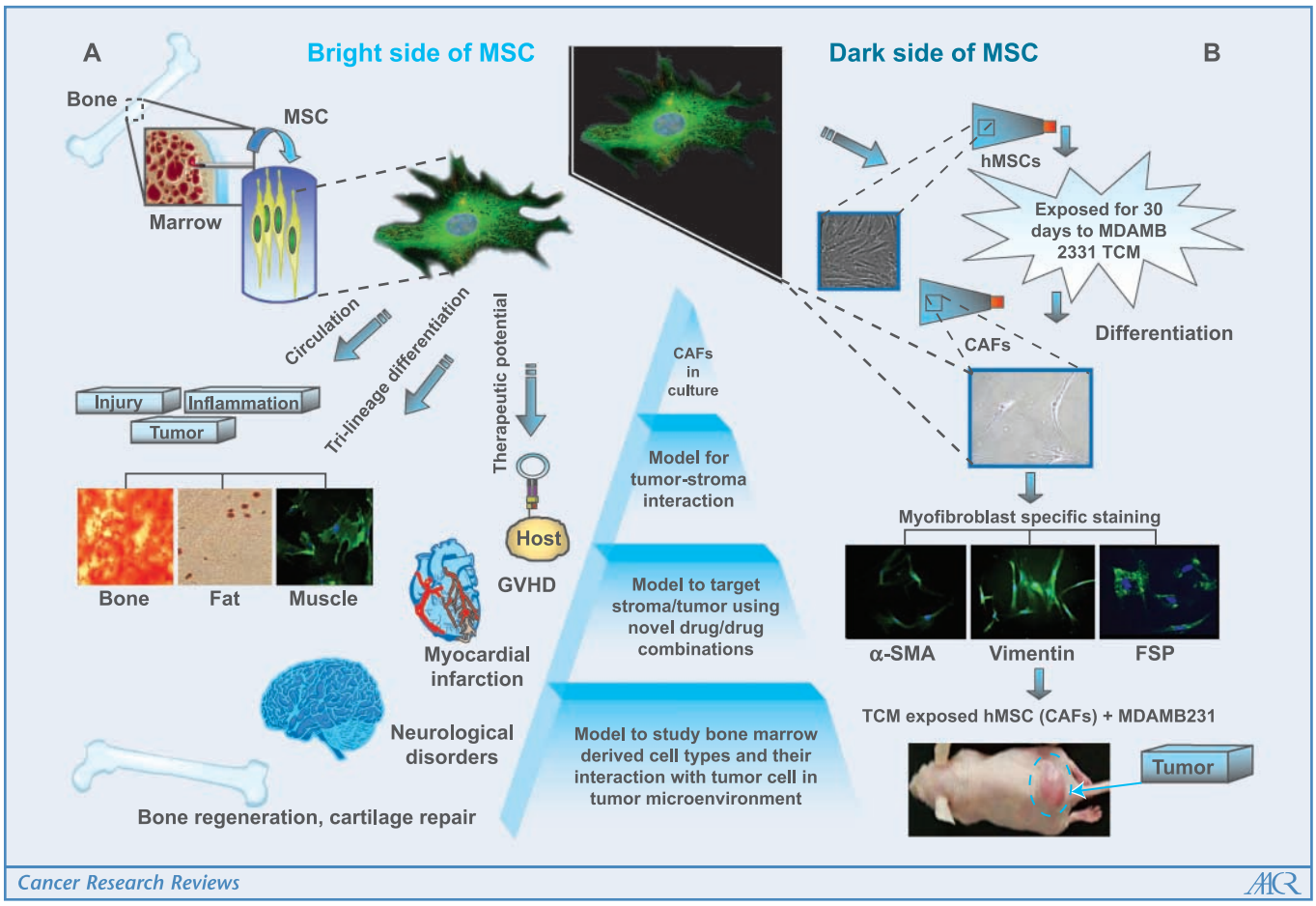

Figure 1. Model depicts two distinct aspects of bone marrow–derived MSCs. *A,* the bright side of MSCs: MSCs migrate toward injury, tumor, and inflammatory sites. MSCs also possess differentiation and immunomodulatory functions, which are being further explored for various regenerative therapies; MSCs are in clinical trials for myocardial infarction and graft-versus-host disease (*GVHD*). *B,* the dark side of MSCs: When cultured in conditioned medium derived from cancer cell lines for prolonged periods, MSCs assume CAF-like properties. These activated MSCs have increased expression of CAF markers such as α-SMA, vimentin, and fibroblast surface protein and, in addition, support growth of breast cancer cells *in vitro* as well as *in vivo* in a xenograft model.

genomic alterations in stromal cells of a microenvironment of a tumor may contribute to clinical outcome. In an analysis of somatic and stromal cell mutations, the authors showed that stroma-specific loss of heterozygosity or allelic imbalance was associated with somatic TP53 mutations and regional lymph node metastases in sporadic breast cancer but not in hereditary breast cancer. The issue of whether the identified mutations correlate with stromal changes or whether this is a reflection of methodologic artifacts has been raised and larger studies on tumor-associated stromal cells from tumor specimens may be helpful in settling this issue.

In an earlier study, the same group investigated whether the apparently nonmalignant stroma surrounding the tumor epithelium can acquire genomic alterations and contribute to cancer initiation and progression in head and neck squamous cell carcinoma (SCC). Tumor-associated stroma of head and neck SCC from smokers was found to have a high degree of genomic alterations. The results suggested that stroma-specific genetic alterations were possibly associated with smoking-related head and neck SCC genesis (21).

It has become clear that the initiation and progression of carcinomas depend not only on alterations in tumor epithelial cells but also on changes in their microenvironment. To study changes in stromal cells within the tumor microenvironment, Hasawi and colleagues (22) undertook to characterize CAFs and their tumor counterpart fibroblasts (TCF) at the cellular and molecular level in a small subset of breast cancer patients using normal breast fibroblasts (NBF) from plastic surgery as a control. The results suggested that the p53/p21 response to γ-radiation was attenuated in 70% of CAFs, whereas it was normal in all of the TCF and NBF cells. These results indicate that alterations in the p53 pathway can occur in breast CAFs and their corresponding adjacent counterparts, further pointing to the important role that stroma may play in breast carcinogenesis and treatment.

Although alterations in the tumor suppressor p53 have been reported in tumor-associated stromal cells, the consequence of these alterations is not clearly understood. Dudley and colleagues (23) have investigated p53 status and response to p53-activating drugs using tumor-associated stromal cells from A375 melanoma and PC3 prostate carcinoma xenografts, as well as from a spontaneous prostate tumor model (TRAMP). Unlike normal stromal cells, tumor-associated stromal cells failed to arrest in G_2 after etoposide treatment, failed to up-regulate p53-inducible genes, and failed to undergo apoptosis after treatment with vincristine. Tumor-associated stromal cells were also found to be less sensitive to p53-activating drugs. Knockdown of p53 in normal stromal cells produced similar results strongly supporting the contention that there was loss of p53 response in tumor-associated stromal cells.

Recent investigation into the role of tumor-associated stromal cells in supporting aerobic glycolysis (also called the Warburg effect) has revealed that activation of highly conserved mammalian uncoupling proteins through interaction with the microenvironment may facilitate the Warburg effect in the absence of permanent respiratory impairment (24). Additionally, tumor-associated stromal cells have been reported to cooperate with tumor cells by taking up the lactate produced by tumor cells and secreted into the microenvironment. The stromal cells take up lactate via monocarboxylate transporters (MCT1 and MCT2) and, after conversion to pyruvate, secrete it into the extracellular milieu where it can be used by local cell constituents for oxidative phosphorylation (25, 26).

Although little is known regarding how changes in stromal gene expression affect epithelial tumor progression, it is increasingly evident that cancer is influenced by signals emanating from tumor stroma. In a recent report, Finak and colleagues (27), by studying gene expression profiles of tumor stroma from 53 primary breast tumors obtained by laser capture microdissection, were able to generate a novel stroma-derived prognostic predictor (SDPP) capable of stratifying disease outcome independently of standard clinical prognostic factors. The SDPP signature was able to predict outcome in several previously published tumor-derived expression data sets, to identify poor-outcome individuals from multiple clinical subtypes, including lymph node–negative tumors. The SDPP was shown to be an improved prognostic predictor compared with previously published methods, particularly for HER2-positive breast tumors. Genes showing strong prognostic tendencies included those associated with differential immune responses and angiogenic and hypoxic responses underscoring the importance of stromal biology in tumor progression.

The plasticity of both the epithelial tumor cells and bone marrow–derived MSCs and its effect on tumor biology remain a subject of intense investigation. On the one hand, tumor cells are known to undergo epithelial mesenchymal transition (28, 29), whereas on the other hand, MSCs are also capable of mesenchymal epithelial transition (30), thus adding to the complexity of cell types in the microenvironment.

Implications of MSCs: A Model to Study Tumor Stroma Interactions

In vitro and coimplantation models combining tumor cells and hMSCs hold great promise as a system in which the interaction between tumor and stroma can be manipulated and studied. Additionally, this may provide a cell culture method for generating one of the important cell types of the tumor stroma, the activated myofibroblasts.

A better understanding of the interplay between different bone marrow–derived cell types and the tumor cells within the tumor microenvironment will be important in developing strategies for improved tumor therapy that take into account the influence of the microenvironment on tumor survival and growth. Another important player in the tumor microenvironment is the tumor-associated macrophage (TAM). To functionally reconstitute the tumor microenvironment *in vitro*, it will be important to include TAMs in the cellular mixture. We have initiated studies to model the tumor-stroma interaction *in vitro* by culturing TCM-exposed hMSCs (representing CAFs), luciferase-expressing tumor cells (to report growth of tumor cells in the three cell mix), and differentiated HL60/U937 cells as surrogates for TAM. The presence of CAFs and TAMs promotes growth of tumor cells as measured by increased luciferase activity.[5] It is now becoming clearer that CAFs and TAMs actively participate in altering the growth and drug response of tumors *in vivo* (31–33). MSCs, which have very high levels of asparaginase expression, can protect leukemic cells from asparaginese cytotoxicity by providing increased concentrations of asparagine in the leukemic cell microenvironment (31).

This system can also be used to study the contribution of freshly harvested tumor-associated fibroblasts from dissected tumors on the growth of a similar type of tumor cell. By establishing a panel of luciferase-expressing tumor cells representing a variety of tumor types and subtypes, one can effectively assess the influence of specific tumor-associated fibroblasts on tumor growth. By further engineering the luciferase system, one can envision specific reporters for pathways that may be activated in different tumors; for example, one can study whether androgen-independent prostate cancer cells are still influenced by the tumor stroma. The relative ease with which the reconstituted tumor in its microenvironment can be transplanted as a xenograft may also permit more relevant drug response studies *in vivo*.

Another important aspect of the reconstituted tumor in its microenvironment is the ability to evaluate chemopreventive measures both *in vitro* and *in vivo*. By pretreating any or all of the components of the reconstituted tumor and its microenvironment, one can, in principle, rank chemopreventive agents by potency as well as cell type specificity.

More innovative use of the reconstituted system may include study of gap junctions and other direct communication means between tumor cells and other cell types. One can also add in pericytes, endothelial progenitor cells, and lymphocytes to complete the picture of the tumor microenvironment.

This type of experimental system provides a more complete recapitulation of an *in vivo* solid tumor, and may provide a more realistic model for investigation of tumor biology as well as chemosensitivity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143–7.
2. Fox JM, Chamberlain G, Ashton BA, Middleton J. Recent advances into the understanding of mesenchymal stem cell trafficking. Br J Haematol 2007;137:491–502.
3. Picinich SC, Mishra PJ, Mishra PJ, Glod J, Banerjee D. The therapeutic potential of mesenchymal stem cells. Expert Opin Biol Ther 2007;7:965–73.
4. Mendes SC, Robin C, Dzierzak E. Mesenchymal progenitor cells localize within hematopoietic sites throughout ontogeny. Development 2005;132:1127–36.
5. Sordi V, Malosio ML, Marchesi F, et al. Bone marrow mesenchymal stem cells express a restricted set of functionally active chemokine receptors capable of promoting migration to pancreatic islets. Blood 2005;106:419–27.
6. Giordano A, Galderisi U, Marino IR. From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells. J Cell Physiol 2007;211:27–35.
7. Studeny M, Marini FC, Dembinski JL, et al. Mesenchymal stem cells: potential precursors for tumor

stroma and targeted-delivery vehicles for anticancer agents. J Natl Cancer Inst 2004;96:1593–603.
8. Studeny M, Marini FC, Champlin RE, et al. Bone marrow-derived mesenchymal stem cells as vehicles for interferon-β delivery into tumors. Cancer Res 2002;62: 3603–8.
9. Khakoo AY, Pati S, Anderson S, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. J Exp Med 2003; 203:1235–47.
10. Djouad F, Plence P, Bony C, et al. Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. Blood 2003;102:3837–44.
11. Djouad F, Bony C, Apparailly F, et al. Earlier onset of syngeneic tumors in the presence of mesenchymal stem cells. Transplantation 2006;82:1060–6.
12. Psaltis PJ, Zannettino A, Worthley SG, Gronthos S. Mesenchymal stromal cells - potential for cardiovascular repair. Stem Cells 2008;26:2201–10. Epub 2008 Jul 3.
13. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease. Nat Rev Immunol 2008;8: 726–36.
14. Bissell MJ, Radisky D. Putting tumors in context. Nat Rev Cancer 2001;1:46–54.
15. Orimo A, Gupta PB, Sgroi DC, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 2005;121:335–48.
16. Direkze NC, Hodivala-Dilke K, Jeffery R, et al. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res 2004;64:8492–5.
17. Mishra PJ, Mishra PJ, Humeniuk R, et al. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. Cancer Res 2008;68: 4331–9.
18. Karnoub AE, Dash AB, Vo AP, et al. Mesenchymal stem cells within tumor stroma promote breast cancer metastasis. Nature 2007;449:557–63.
19. Guo X, Oshima H, Kitmura T, et al. Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer. J Biol Chem 2008;283: 19864–71.
20. Patocs A, Zhang L, Xu Y, et al. Breast-cancer stromal cells with TP53 mutations and nodal metastases. N Engl J Med 2007;357:2543–51.
21. Weber F, Xu Y, Zhang L, et al. Microenvironmental genomic alterations and clinicopathological behavior in head and neck squamous cell carcinoma. JAMA 2007; 297:187–95.
22. Hawsawi NM, Ghebeh H, Hendrayani SF, et al. Breast carcinoma-associated fibroblasts and their counterparts display neoplastic-specific changes. Cancer Res 2008;68: 2717–25.
23. Dudley AC, Shih SC, Cliffe AR, Hida K, Klagsbrun M. Attenuated p53 activation in tumor-associated stromal cells accompanies decreased sensitivity to etoposide and vincristine. Br J Cancer 2008;99:118–25.
24. Samudio I, Fiegl M, McQueen T, Clise-Dwyer K, Andreeff M. The Warburg effect in leukemia-stroma cocultures is mediated by mitochondrial uncoupling associated with uncoupling protein-2 activation. Cancer Res 2008;68:5198–205.
25. Koukourakis MI, Giatromanolaki A, Harris AL, Sivridis E. Comparison of metabolic pathways between cancer cells and stromal cells in colorectal carcinomas: a metabolic survival role for tumor-associated stroma. Cancer Res 2006;66:632–7.
26. Kroemer G, Pouyssegur J. Tumor cell metabolism: cancer's Achilles' heel. Cancer Cell 2008;13:472–82.
27. Finak G, Bertos N, Pepin F, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med 2008;14:518–27.
28. Salomon AV, Thiery JP. Host microenvironment in breast cancer development Epithelial-mesenchymal transition in breast cancer development. Breast Cancer Res 2003;5:101–6.
29. Radisky DC. Epithelial-mesenchymal transition. J Cell Sci 2005;118:4325–6.
30. Chaffer CL, Brennan JP, Slavin JL, et al. Mesenchymal-to-epithelial transition facilitates bladder cancer metastasis: role of fibroblast growth factor receptor-2. Cancer Res 2006;66:11271–8.
31. Iwamoto S, Mihara K, Dowining JR, Pui C-H, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia to asparaginase. J Clin Invest 2007;117:1049–57.
32. Mantovani A, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. Cancer Metastasis Rev 2006; 25:315–22.
33. Luo Y, Zhou H, Krueger J, et al. Targeting tumor-associated macrophages as a novel strategy against breast cancer. J Clin Invest 2006;116:2132–41.