direct contact between BM-derived MSCs (BM-MSCs) and gastric cancer cells in vitro increases the proportion of CD133-positive gastric cancer cells. This mechanism is thought to involve stimulation of Wnt-5a and transforming growth factor (TGF)-β by BM-BMCs, directly affecting the gastric cancer cells. Moreover, direct contact between BM-MSCs and gastric cancer cells increases the expression of vimentin and Snail, which is a transcription factor that mediates epithelial-mesenchymal transitions (EMT). Human BM-MSCs can enhance the proliferation, invasion, and chemoresistance of gastric cancer cells. The regulatory mechanism involved is likely associated with increased expression of stem cell markers and EMT-related factors in gastric cancer cells. CD271, which is a marker of BM-MSCs, is expressed in scirrhous carcinoma, and the expression of Wnt-5a and TGF-β in cancer cells is up-regulated.

BM-MSCs provide an advantageous tumor microenvironment for gastric cancer. Moreover, BM-MSC-related molecules may be considered biomarkers of gastric cancer.

Key words: Bone Marrow; Mesenchymal Stem Cells; Epithelial-Mesenchymal Transition; Gastric Cancer; Cancer Stem Cells

ABSTRACT

Tumorigenesis is driven by alterations in tumor cells and also changes in the stromal microenvironment. Mesenchymal stem cells (MSCs) are promising candidates for the treatment of various tumors. MSCs are unlikely to be rejected by the immune system, could potentially home to the tumor site, and may interact with tumor cells to influence their growth and metastasis. Using mouse models of inflammation-induced gastric cancer, at least 20% of cancer-associated fibroblasts were found to originate from bone marrow (BM) and were derived from MSCs. Direct contact between BM-derived MSCs (BM-MSCs) and gastric cancer cells in vitro increases the proportion of CD133-positive gastric cancer cells. This mechanism is thought to involve stimulation of Wnt-5a and transforming growth factor (TGF)-β by BM-BMCs, directly affecting the gastric cancer cells. Moreover, direct contact between BM-MSCs and gastric cancer cells increases the expression of vimentin and Snail, which is a transcription factor that mediates epithelial-mesenchymal transitions (EMT). Human BM-MSCs can enhance the proliferation, invasion, and chemoresistance of gastric cancer cells. The regulatory mechanism involved is likely associated with increased expression of stem cell markers and EMT-related factors in gastric cancer cells. CD271, which is a marker of BM-MSCs, is expressed in scirrhous carcinoma, and the expression of Wnt-5a and TGF-β in cancer cells is up-regulated. BM-MSCs provide an advantageous tumor microenvironment for gastric cancer. Moreover, BM-MSC-related molecules may be considered biomarkers of gastric cancer.

INTRODUCTION

During tumor progression, epithelial-mesenchymal transitions (EMT) contribute considerably to the malignant characteristics of tumors, including local invasion and distant metastasis. EMT is the key phenomenon that tightly regulates the stem cell-like characteristics of both normal and malignant cells. Since cancer stem cells (CSCs) in solid cancers were first identified in the early half of the 2000s,
establishment of a treatment targeting CSCs to achieve a radical cure of cancer has become an important goal. Mesenchymal stem cells (MSCs) are defined as pluripotent stem cells that contribute to normal bone, adipose, cartilage, and muscle[5]. MSCs were first isolated from the bone marrow (BM), adipose tissue, synovial tissue, lung tissue, umbilical cord blood, and peripheral blood[1-4], and are now commonly isolated from almost every organ of adult mice[41], laying the foundation for widespread experimental research into MSCs. MSCs show strong tropism towards different types of solid tumors, including prostate carcinoma[34,35], breast cancer[41-43], colon cancer[44], lung cancer[45], ovarian cancer[46,47], and gliomas[48]. MSC-like cells can be isolated from tumors such as bone sarcomas, lipomas, and gastric cancers[23-28]. The characteristics of these MSC-like cells, with respect to morphology, gene expression, surface antigen expression, and differentiation potential, are similar to those of BM-derived MSCs (BM-MSCs).

MSCs have received widespread attention because they are able to migrate and engraft into tumors. In addition, MSCs regulate the growth, invasion, and metastasis of gastric cancer cells[29-31]. MSCs enhance gastric cancer metastatic dissemination via the activation of EMT[29,30]. MSCs derived from the gastric submucosa can transform into cancer-associated fibroblasts (CAFs) and endothelial cells, which promote gastric cancer progression[31]. The characteristics of these MSC-like cells, with respect to morphology, gene expression, surface antigen expression, and differentiation potential, are similar to those of BM-derived MSCs (BM-MSCs).

MSCs exposed to tumor-conditioned medium assume a CAF-like phenotype, including sustained expression of stroma cell-derived factor 1 (SDF-1) and the ability to promote tumor cell growth[32]. Quanté et al[33] showed that at least 20% of CAFs originate from the BM and are derived from MSCs. MSCs originate in the BM but can be found throughout the body; they are often involved in tissue remodeling after injury or chronic inflammation. BM-derived cells are often recruited to carcinogenic sites by cytokines such as interleukin (IL)-1[34,35], and CAFs promote further cell recruitment through secretion of chemokines such as SDF-1[36]. MSCs are among the BM-derived cells that are recruited to tumors and that promote their growth. Some studies have suggested that MSCs can differentiate into CAFs[37].

In this review article, we discuss data showing that BM-MSCs provide an advantageous tumor microenvironment for EMT and the restoration of gastric cancer stem cells.

THE ROLE OF BM-MSCs AS CAFs

In the tumor microenvironment, immune cells, CAFs, various blood and lymphatic cells, and MSCs are present. These cells engage in cross-talk with tumor cells and secrete chemokines, growth factors, and matrix metalloproteases that likely promote the proliferation and invasion of tumor cells[28,41]. Although tumorigenesis is widely considered to be regulated by interactions between tumor cells and CAFs, the precise origin and function of CAFs are unclear. Kabashima-Niibe et al[42] focused on the role of α-smooth muscle actin (SMA)-positive myofibroblast-like cells in EMT regulation and cancer progression of pancreatic cancer cells. Their results indicated that α-SMA-positive myofibroblast-like cells are enriched in areas in which cancer cells had undergone EMT, and were frequently identified in human pancreatic cancer specimens[42]. α-SMA-positive cells originating from BM enhance tumor formation by pancreatic cancer cell lines[43].

Pretreatment with transforming growth factor (TGF)-β induces MSCs to express certain molecules, including α-SMA, tenasin C, and podoplanin, which are enriched in the cancer stroma[44]. α-SMA-positive myofibroblast-like cells play an important role in progression of pancreatic cancer, especially in regulating the EMT status and tumor-initiating stem cell-like characteristics[29,32,36,44]. Because MSCs migrate from the BM into tumor tissue[37,38,40], BM-MSCs may be a potential source of α-SMA-positive cells within the tumor stroma.

Tumorigenesis is driven by alterations in tumor cells and also changes in the stromal microenvironment. Using mouse models of inflammation-induced gastric cancer, Quanté et al[30] showed that at least 20% of CAFs originate from BM and are derived from MSCs. α-SMA-positive myofibroblasts are nitch cells that are normally present in BM and increase markedly during cancer progression[31]. CAFs are generated from MSCs and are recruited to the tumor in a TGF-β- and SDF-1α-dependent manner[1]. Therefore, carcinogenesis involves expansion and relocation of BM-nichie cells to the tumor to create a niche that can sustain cancer progression.

MSCs REGULATE EMT AND TUMOR PROGRESSION

EMT is a biological process in which epithelial cells transform into cells with a mesenchymal phenotype. These cells play significant roles in embryonic development, chronic inflammation, tissue

Figure 1 Schematic drawing that depicts interactions between the BM niche (left) and the gastric cancer stroma (right). A significant portion of CAFs originates from BM and is derived from MSCs. The normal BM niche consists of self-renewing MSCs that give rise to MFs that resemble CAFs. MSCs express SDF-1. TGF-β can induce differentiation of MSCs into MFs through an SDF1α-dependent pathway that involves DNA hypomethylation. With cancer progression, the number of CAFs increases markedly in the BM niche and blood. MSC-derived CAFs that are recruited to the dysplastic stomach express IL-6, Wnt-5a, and BMP4, show DNA hypomethylation, and promote tumor growth[31]. Moreover, CAFs are generated from MSCs and are recruited to the tumor in a TGF-β- and SDF-1α-dependent manner[1] (Figure 1). Therefore, carcinogenesis involves expansion and relocation of BM-nichie cells to the tumor to create a niche that can sustain cancer progression.
reconstruction, cancer initiation, and metastasis. During EMT, epithelial cells lose certain characteristics, including polarity and connection with the basement membrane[k], and develop a mesenchymal phenotype, migrating away from their original epithelial layer. This process is accompanied by decreased expression of adhesion molecules, such as E-cadherin, and increased expression of the cytoskeletal protein vimentin and the extracellular matrix protein N-cadherin.

Previous findings suggest that aberrant activation of EMT leads to gastric cancer invasion and metastasis, along with the acquisition of chemoresistance[l]. In the chronic gastritis microenvironment associated with Helicobacter pylori, MSCs that express IL-6, IL-8, and platelet-derived growth factor (PDGF) differentiate into CAFs and induce EMT in GES-1 gastric epithelial cells[k]. Wang et al[k] found that human MSCs enhance the proliferation, invasion, and chemoresistance of gastric cancer cells. The regulatory mechanism involved is likely associated with increased expression of stem cell markers and EMT-related factors in gastric cancer cells[l].

Many chemokines, growth factors, miRNAs, and exosomes derived from MSCs provide a microenvironment that is suitable for the proliferation and invasion of gastric cancer cells. MSCs that secrete soluble signaling molecules induce the expression of vascular endothelial growth factor and the activation of RhoA-GTPase and ERK1/2 in SGC-7901 cells. In addition, they potentiate gastric cancer growth[k]. Huang et al[k] demonstrated a paracrine role for MSCs in promoting gastric cancer growth. Gastric Cancer MSCs that secrete PDGF-DD promote the progression of gastric cancer. Additionally, CCL-5 derived from human BM-MSCs enhances the mobility of human gastric cancer cells via activation of the Src/Cas/paxillin signaling pathway[l]. Other researchers have shown that MSCs can confer a CAF phenotype to gastric cancer cells, induce EMT, and facilitate tumor growth and metastasis through paracrine cues and close physical connections. Exosomes derived from BM-MSCs promote gastric cancer growth and enhance vascular endothelial growth factor expression. Wang et al[k] showed that gastric cancer MSCs promote gastric cancer progression by transferring exosomal miRNAs to gastric cancer cells. BM-MSCs provide an advantageous tumor microenvironment, thereby supporting the reacquisition and maintenance of gastric CSCs[l].

THE EFFECT OF BM-MSCs ON GASTRIC CSCs

After CSCs in solid cancers were first reported in the early half of the 2000s[k,k,k], establishment of a treatment targeting these cells in hopes of a radical cure of cancer has become an important goal. Therefore, research into markers to isolate CSCs and characterize cells isolated with these markers has been active throughout the world.

CD133 is a 120-kDa glycoprotein with five transmembrane domains and is a CSC marker. Despite various theories having been proposed, the biological function of CD133 is still not well understood. Originally, CD133 was identified as a surface marker of hematopoietic stem cells and progenitor cells, but CD133 has also recently been shown to be a marker of CSCs in solid cancers such as brain tumors[l], lung cancer[l], liver cancer[l], colon cancer[l,k,k], pancreatic cancer[l], and prostate cancer[l]. In addition, in lung cancer[l], breast cancer[l], hepatocellular carcinoma[l], colon cancer[l], and pancreatic cancer[l], CD133 expression is strongly related not only to tumor progression, but also to treatment resistance. Gastric cancer cells that express CD133 in the cytoplasm have high potential for malignancy, and this phenotype is associated with cancer progression, chemotherapy resistance, recurrence, and poor prognosis. Cytoplasmic expression of CD133 may be a useful prognostic marker in gastric cancer[l].

To investigate the relationship between cancer cells and BM-MSCs, a co-culture was established with direct contact between MKN-7 cells, which originate from human differentiated gastric adenocarcinoma, and UE6E7T-12 cells, which originate from human BM-MSCs. During co-culture, the MKN-7 cells had increased expression of vimentin and Snail, which is a transcription factor that mediates EMT. Simultaneously, the MKN-7 cells also exhibited increased expression of CD133, which is a marker of CSCs. CD133-positive MKN-7 cells were observed near UE6E7T-12 cells[l]. These results suggested that BM-MSCs induce EMT of gastric cancer cells and increase the number of CSCs. In an in vivo study, the size of the tumor after subcutaneous co-injection of MKN-7 cells and UE6E7T-12 cells into mice was significantly larger than that of the tumor after injection of MKN-7 cells only. An increased number of fibroblasts that originated from UE6E7T-12 cells was observed after co-injection of a mixture of MKN-7 and UE6E7T-12 cells[l]. These results suggest that BM-MSCs contribute to tumor formation as CAFs.

In another in vivo study, CD133-positive MKN-7 cells were co-localized along with BM-MSCs, and direct contact between MKN-7 cells and UE6E7T-12 cells was thought to contribute to tumor formation. Moreover, MKN-7 cells express CD10, MUC2 (which is a large intestinal epithelial marker), villin (which is a small intestinal marker), and chromogranin A (which is a marker of neuroendocrine differentiation)[]. These results suggested that BM-MSCs accelerate the effects of CSCs on gastric cancer cells in multiple ways.

WNT-5a AND GASTRIC CANCER

Wnt proteins constitute a large family of cysteine-rich secreted molecules[]. At least 19 Wnt members have been identified in mammals to date. Wnt family members exhibit unique expression patterns and distinct functions in development and can be divided into three distinct types based on their ability to induce transformation of the mouse mammary epithelial cell line C57MG[].

The intracellular signaling pathway activated by Wnt proteins was originally identified as a β-catenin-dependent signaling pathway that is highly conserved among species[]. According to the most widely accepted current model of the β-catenin pathway, the absence of the Axin complex results in degradation of β-catenin by the proteasome[]. Consequently, the cytoplasmic β-catenin level is low. When Wnt binds to its cell surface receptor (consisting of Frizzled and lipoprotein receptor-related protein 5/6), β-catenin escapes from degradation in the Axin complex[]. The accumulated β-catenin is translocated to the nucleus, where it binds to the transcription factor T-cell factor/lymphoid enhancer factor and thereby stimulates the expression of various genes[]. The Wnt proteins that show high transforming activity in C57MG cells are thought to activate the β-catenin pathway.

Some Wnt proteins activate a β-catenin-independent pathway that primarily modulates cell movement and polarity[]. Wnt-5a is a representative Wnt protein that activates the β-catenin-independent pathway. Some reports indicate that Wnt-5a acts as a tumor suppressor because Wnt-5a has the ability to inhibit the β-catenin pathway[]. On the other hand, the Wnt-5a mRNA level is up-regulated in lung cancers, prostate cancers, and breast cancers[]. A correlation has been observed between Wnt-5a expression and increased cell motility and invasiveness of melanoma cells and breast cancer cells along with tumor-associated macrophages[].
The Role of BM-MSCs in Gastric Cancer

Figure 2 The effect of BM-MSCs on cancer cells. EMT and recovery of stem cell properties of gastric cancer cells induced by direct contact with BM-MSCs are mediated by high Wnt and TGF-β signaling. BM-MSCs: bone marrow-derived mesenchymal stem cells, EMT: epithelial-mesenchymal transition.

Kurayoshi et al. [84] clarified how Wnt-5a is involved in the aggressiveness of gastric cancer and reported that expression of Wnt-5a is correlated with an advanced stage and poor prognosis of gastric cancer. Wnt-5a can stimulate cell migration and invasion by gastric cancer cells. Wnt-5a activates focal adhesion kinase and the small GTP-binding protein Rac, both of which play a role in cell migration. Cell migration, membrane ruffling, and turnover of paxillin are suppressed in Wnt-5a knock-down cells. Furthermore, anti-Wnt-5a antibody suppresses gastric cancer cell migration [84].

TGF-β PATHWAY

Although TGF-β suppresses proliferation of certain carcinoma cells and is a well-known tumor suppressor, it promotes proliferation of tumors of non-epithelial origin, including gliomas and osteosarcomas, through induction of PDGF-BB [85,86]. TGF-β binds to type I and type II serine/threonine kinase receptors and transduces intracellular signals principally through Smad proteins [87-89]. Upon phosphorylation by type I receptors, receptor-regulated Smads (R-smad2 and -3) form heteromeric complexes with common-partner Smad (Co-Smad; Smad4), translocate into the nucleus, and regulate expression of various target genes. In addition to induction of proliferation, the TGF-β pathway has also been implicated in invasion, metastasis, and intratumoral angiogenesis of gliomas. These multiple roles for TGF-β in glioma progression have promoted the development of therapeutic agents based on inhibition of the TGF-β pathway [90].

TGF-β directly induces Sox4 expression. Subsequently, Sox4 promotes expression of Sox2, which plays a significant role in sustaining the stem cell phenotype of glioma-initiating cells, possibly in cooperation with other signaling pathways. A TGF-β inhibitor blocks this “TGF-β-Sox4-Sox2” pathway, promotes differentiation of glioma-initiating cells, and inhibits their aggressiveness [91].

WNT-5A IN CANCER CELLS AND TGF-B1 IN BM-MSCS

After co-culture and direct contact between MKN-7 cells and UE6E7T-12 cells, the expression of Wnt-5a in MKN-7 cells and TGF-β1 in BM-MSCs is induced, and the concentration of TGF-β1 in the culture medium increases. The CD133 mRNA level and the proportion of CD133-positive MKN-7 cells both significantly increase after treating CD133-negative MKN-7 cells with Wnt-5a. Similarly, TGF-β1 significantly increases the CD133 mRNA level and the percent of CD133-positive MKN-7 cells. Co-culture of MKN-7 and UE6E7T-12 cells increases not only the expression of Wnt-5a and TGF-β1, but also β-catenin and Smad4, which are part of the Wnt-5a and TGF-β1 signaling pathway. A Wnt-5a inhibitor and a TGF-β1 inhibitor both interrupt translocation of β-catenin and Smad4 into the nucleus and suppress the recovery of CD133-positive MKN-7 cells [67]. These results suggested that direct contact between BM-MSCs and cancer cells increases the expression of Wnt-5a in cancer cells and promotes the secretion of TGF-β1 from BM-MSCs. Autocrine activity of Wnt-5a and paracrine activity of TGF-β1 are thought to induce the stem cell phenotype of cancer cells (Figure 2).
and metastatic activity of gastric cancer cells, and promotion of the transition to the scirrhous type of gastric cancer\(^{20}\). These results suggest that EMT and acquisition of stem cell properties in gastric cancer cells induced by direct contact with BM-MSCs are mediated by high activity of Wnt and TGF-\(\beta\), which are important for the process of proliferation and progression of gastric cancer (Figure 1). In the future, Wnt-5a and TGF-\(\beta\) may become important therapeutic targets.

**CONCLUSION**

MSCs play a significant role in the tumorigenesis and development of gastric cancer. MSCs significantly promote the growth of gastric tumors. The general mechanism involved is likely associated with paracrine cytokines such as TGF-\(\beta\) in BM-MSCs and autocrine cytokines such as Wnt-5a in gastric cancer cells after cell-cell contact, followed by increased expression of stem cell markers in gastric cancer cells and induction of EMT. MSCs provide an advantageous tumor microenvironment, and MSC-related molecules may be considered biomarkers of gastric cancer.

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