

# Bone Marrow-Derived Mesenchymal Stem Cells Regulate Epithelial-Mesenchymal Transitions and Gastric Cancer Stem Cells

Keishiro Aoyagi, Junya Kizaki, Taro Isobe, Taizan Minami, Yoshito Akagi

Keishiro Aoyagi, Junya Kizaki, Taro Isobe, Taizan Minami, Yoshito Akagi, Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka, 830-0011, Japan

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**Correspondence to:** Keishiro Aoyagi, MD, PhD, Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka, 830-0011, Japan

**Email:** [keishiro@med.kuurume-u.ac.jp](mailto:keishiro@med.kuurume-u.ac.jp)

**Telephone:** +81-942-35-3011

**Fax:** +81-943-34-0709

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## 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)- $\beta$  by BM-MSCs, 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- $\beta$  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

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## INTRODUCTION

During tumor progression, epithelial-mesenchymal transitions (EMT) contribute considerably to the malignant characteristics of tumors, including local invasion and distant metastasis<sup>[1,2]</sup>. EMT is the key phenomenon that tightly regulates the stem cell-like characteristics of both normal and malignant cells<sup>[3,4]</sup>. 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<sup>[5]</sup>. MSCs were first isolated from the bone marrow (BM), adipose tissue, synovial tissue, lung tissue, umbilical cord blood, and peripheral blood<sup>[5-10]</sup>, and are now commonly isolated from almost every organ of adult mice<sup>[11]</sup>, laying the foundation for widespread experimental research into MSCs.

MSCs show strong tropism towards different types of solid tumors, including prostate carcinoma<sup>[12,13]</sup>, breast cancer<sup>[14,15]</sup>, colon cancer<sup>[16]</sup>, lung cancer<sup>[17]</sup>, ovarian cancer<sup>[18,19]</sup>, and gliomas<sup>[20]</sup>. MSC-like cells can be isolated from tumors such as bone sarcomas, lipomas, and gastric cancers<sup>[21-24]</sup>. 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<sup>[25-28]</sup>. MSCs enhance gastric cancer invasion and metastatic dissemination via the activation of EMT<sup>[29,30]</sup>. MSCs derived from the gastric submucosa can transform into cancer-associated fibroblasts (CAFs) and endothelial cells, which promote gastric cancer progression<sup>[31]</sup>. Several studies have pointed to MSCs as a potential source of CAFs<sup>[32]</sup>. MSCs, when mixed with weakly metastatic human breast carcinoma cells, increase the metastatic capabilities of the cancer cells<sup>[33,34]</sup>.

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<sup>[35]</sup>. Quante *et al.*<sup>[36]</sup> 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 $\beta$ <sup>[37,38]</sup>, and CAFs promote further cell recruitment through secretion of chemokines such as SDF-1<sup>[39]</sup>. 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<sup>[40]</sup>.

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<sup>[24,41]</sup>. 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.*<sup>[42]</sup> focused on the role of  $\alpha$ -smooth muscle actin (SMA)-positive myofibroblast-like cells in EMT regulation and cancer progression of pancreatic cancer cells. Their results indicated that  $\alpha$ -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<sup>[42]</sup>.  $\alpha$ -SMA-positive cells originating from BM enhance tumor formation by pancreatic

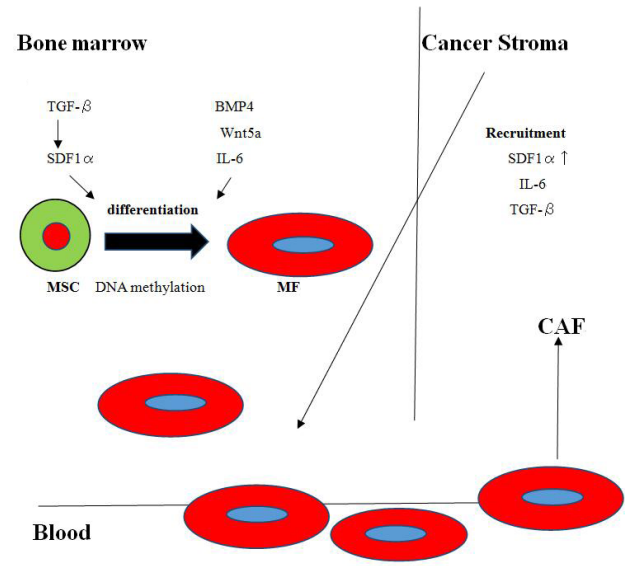
cancer cell lines<sup>[36]</sup>.

Pretreatment with transforming growth factor (TGF)- $\beta$  induces MSCs to express certain molecules, including  $\alpha$ -SMA, tenascin C, and podoplanin, which are enriched in the cancer stroma<sup>[43]</sup>.  $\alpha$ -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<sup>[35,36,44]</sup>. Because MSCs migrate from the BM into tumor tissue<sup>[37,38,45]</sup>, BM-MSCs may be a potential source of  $\alpha$ -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, Quante *et al.*<sup>[36]</sup> showed that at least 20% of CAFs originate from BM and are derived from MSCs.  $\alpha$ -SMA-positive myofibroblasts are niche cells that are normally present in BM and increase markedly during cancer progression<sup>[36]</sup>. MSC-derived CAFs that are recruited to the dysplastic stomach express IL-6, Wnt-5a, and BMP4, show DNA hypomethylation, and promote tumor growth<sup>[36]</sup>. Moreover, CAFs are generated from MSCs and are recruited to the tumor in a TGF- $\beta$ - and SDF-1 $\alpha$ -dependent manner<sup>[36]</sup> (Figure 1). Therefore, carcinogenesis involves expansion and relocation of BM-niche 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- $\beta$  can induce the differentiation of MSCs into MFs through an SDF1 $\alpha$ -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. CAFs are generated from MSCs and are recruited to the tumor in a TGF- $\beta$ - and SDF-1 $\alpha$ -dependent manner. BM: bone marrow; CAFs: carcinoma associated fibroblasts; MSCs: mesenchymal stem cells; MFs: myofibroblasts; SDF1 $\alpha$ : stroma cell-derived factor 1  $\alpha$ ; TGF- $\beta$ : transforming growth factor  $\beta$ ; IL-6: interleukin 6.

reconstruction, cancer initiation, and metastasis. During EMT, epithelial cells lose certain characteristics, including polarity and connection with the basement membrane<sup>[46]</sup>, 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<sup>[47]</sup>. 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<sup>[48]</sup>. Wang *et al.*<sup>[49]</sup> 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<sup>[49]</sup>.

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<sup>[50]</sup>. Huang *et al.*<sup>[51]</sup> 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<sup>[52]</sup>. 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.*<sup>[49]</sup> 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<sup>[49]</sup>.

## THE EFFECT OF BM-MSCs ON GASTRIC CSCs

After CSCs in solid cancers were first reported in the early half of the 2000s<sup>[53,54]</sup>, 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<sup>[54]</sup>, lung cancer<sup>[55]</sup>, liver cancer<sup>[56]</sup>, colon cancer<sup>[57,58]</sup>, pancreatic cancer<sup>[59]</sup>, and prostate cancer<sup>[60]</sup>. In addition, in lung cancer<sup>[61]</sup>, breast cancer<sup>[62]</sup>, hepatocellular carcinoma<sup>[63]</sup>, colon cancer<sup>[64]</sup>, and pancreatic cancer<sup>[65]</sup>, 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<sup>[66]</sup>.

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<sup>[67]</sup>. 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<sup>[67]</sup>. These results suggest that BM-MSCs contribute to tumor formation as CAFs.

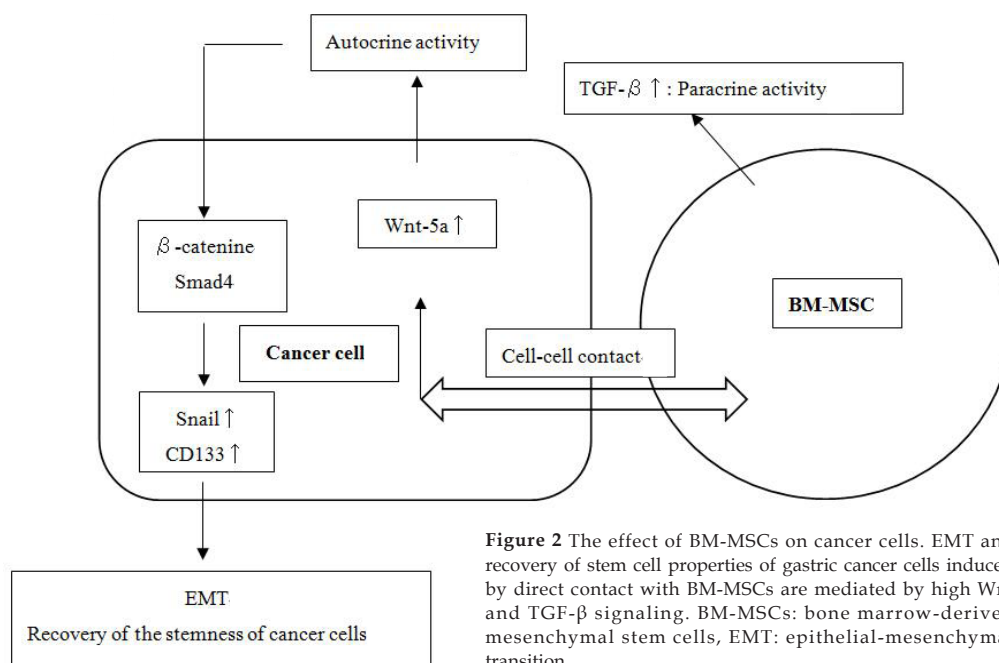
In another *in vivo* study, CD133-positive MKN-7 cells were co-localized along with BM-MSCs, and direct contact between MKN-7 cells and UE6E7T-12 cells was thought to contribute to tumor formation. Moreover, MKN-7 cells express CD10, MUC2 (which is a large intestinal epithelial marker), villin (which is a small intestinal marker), and chromogranin A (which is a marker of neuroendocrine differentiation)<sup>[67]</sup>. 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<sup>[68]</sup>. 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<sup>[69,70]</sup>.

The intracellular signaling pathway activated by Wnt proteins was originally identified as a  $\beta$ -catenin-dependent signaling pathway that is highly conserved among species<sup>[68]</sup>. According to the most widely accepted current model of the  $\beta$ -catenin pathway, the absence of the Axin complex results in degradation of  $\beta$ -catenin by the proteasome<sup>[71-74]</sup>. Consequently, the cytoplasmic  $\beta$ -catenin level is low. When Wnt binds to its cell surface receptor (consisting of Frizzled and lipoprotein receptor-related protein 5/6),  $\beta$ -catenin escapes from degradation in the Axin complex<sup>[75]</sup>. The accumulated  $\beta$ -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<sup>[76]</sup>. The Wnt proteins that show high transforming activity in C57MG cells are thought to activate the  $\beta$ -catenin pathway.

Some Wnt proteins activate a  $\beta$ -catenin-independent pathway that primarily modulates cell movement and polarity<sup>[70,77]</sup>. Wnt-5a is a representative Wnt protein that activates the  $\beta$ -catenin-independent pathway. Some reports indicate that Wnt-5a acts as a tumor suppressor because Wnt-5a has the ability to inhibit the  $\beta$ -catenin pathway<sup>[78-80]</sup>. On the other hand, the Wnt-5a mRNA level is up-regulated in lung cancers, prostate cancers, and breast cancers<sup>[81]</sup>. 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<sup>[82,83]</sup>.



**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- $\beta$  signaling. BM-MSCs: bone marrow-derived mesenchymal stem cells, EMT: epithelial-mesenchymal transition.

Kurayoshi *et al.*<sup>[84]</sup> 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<sup>[84]</sup>.

## TGF- $\beta$ PATHWAY

Although TGF- $\beta$  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<sup>[85,86]</sup>. TGF- $\beta$  binds to type I and type II serine/threonine kinase receptors and transduces intracellular signals principally through Smad proteins<sup>[87-89]</sup>. 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- $\beta$  pathway has also been implicated in invasion, metastasis, and intratumoral angiogenesis of gliomas. These multiple roles for TGF- $\beta$  in glioma progression have promoted the development of therapeutic agents based on inhibition of the TGF- $\beta$  pathway<sup>[90]</sup>.

TGF- $\beta$  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- $\beta$  inhibitor blocks this "TGF- $\beta$ -Sox4-Sox2" pathway, promotes differentiation of glioma-initiating cells, and inhibits their aggressiveness<sup>[91]</sup>.

## WNT-5a IN CANCER CELLS AND TGF- $\beta$ 1 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- $\beta$ 1 in BM-MSCs is induced, and the concentration of TGF- $\beta$ 1 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- $\beta$ 1 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- $\beta$ 1, but also  $\beta$ -catenin and Smad4, which are part of the Wnt-5a and TGF- $\beta$ 1 signaling pathway. A Wnt-5a inhibitor and a TGF- $\beta$ 1 inhibitor both interrupt translocation of  $\beta$ -catenin and Smad4 into the nucleus and suppress the recovery of CD133-positive MKN-7 cells<sup>[67]</sup>. 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- $\beta$ 1 from BM-MSCs. Autocrine activity of Wnt-5a and paracrine activity of TGF- $\beta$ 1 are thought to induce the stem cell phenotype of cancer cells (Figure 2).

## EMT AND STEM CELL PROPERTIES OF GASTRIC CANCER

Expression of Wnt-5a is correlated with advanced stage and poor prognosis in gastric cancer, and Wnt-5a is more frequently expressed in the scirrhous type of gastric cancer than in other types of gastric cancer<sup>[84]</sup>. TGF- $\beta$  induces MSCs to express certain molecules, including  $\alpha$ -SMA, tenascin C, and podoplanin, which are enriched within the cancer stroma<sup>[43]</sup>. TGF- $\beta$  induces EMT in immortalized human mammary epithelial cells and increases their ability to form mammospheres<sup>[4]</sup>. These findings suggest a direct link between EMT and acquisition of epithelial stem cell properties in carcinomas.

CD271, which is a marker of BM-MSCs, is frequently expressed in the scirrhous type of gastric cancer. However, CD271-positive cells are not observed in well-differentiated adenocarcinomas. High, frequent expression of Wnt-5a and the TGF- $\beta$ 1 receptor are observed in the scirrhous type of cancer. Moreover, CD133-positive cells are more frequently found in the scirrhous type of gastric cancer than in other types of gastric cancer. BM-MSCs are thought to play a role in formation of a favorable tumor microenvironment, acquisition of stem cell properties of gastric cancer, enhancement of the invasive

and metastatic activity of gastric cancer cells, and promotion of the transition to the scirrhous type of gastric cancer<sup>[67]</sup>. 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.

## REFERENCES

- Thiery JP. Epithelial-mesenchymal transitions in tumor progression. *Nat Rev Cancer*. 2002; **2**: 442-454. [DOI: 10.1038/nrc822]; [PMID: 12189386]
- Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest*. 2009; **119**: 1420-1428. [DOI: 10.1172/JCI39104]; [PMID: 19487818]
- Morel AP, Lievre M, Thomas C, Hinkal G, Ansieau S, Puisieux A. Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS ONE*. 2008; **3**: e2888. [DOI: 10.1371/journal.pone.0002888]; [PMID: 18682804]; [PMCID: PMC2492808]
- Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell*. 2008; **133**: 704-715. [DOI: 10.1016/j.cell.2008.03.027]; [PMID: 18485877]; [PMCID: PMC272803]
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; **284**: 143-147. [DOI: 10.1126/science.284.5411.143]; [PMID: 10102814]
- Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Benhaim P, Lorenz HP, Hedrick MH. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng*. 2001; **7**: 211-228. [DOI: 10.1089/107632701300062859]; [PMID: 11304456]
- De Bari C, Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. *Arthritis Rheum*. 2001; **44**: 1928-1942. [DOI: 10.1002/1529-0131(200108)44:8<1928::AID-ART331>3.0.CO;2-P
- Sabatini F, Petecchia L, Tavian M, Jodon de Villeroché V, Rossi GA, Brouty-Boyd D. Human bronchial fibroblasts exhibit a mesenchymal stem cell phenotype and multilineage differentiating potentialities. *Lab Invest*. 2005; **85**: 962-971. [DOI: 10.1038/labinvest.3700300]; [PMID: 15924148]
- Erices A, Conget P, Minguell JJ. Mesenchymal progenitor cells in human umbilical cord blood. *Br J Haematol*. 2000; **109**: 235-242. [DOI: 10.1046/j.1365-2141.2000.01986.x]; [PMID: 10848804]
- Zvaifler NJ, Marinova-Mutafchieva L, Adams G, Edwards CJ, Moss J, Burger JA, Maini RN. Mesenchymal precursor cells in the blood of normal individuals. *Arthritis Res*. 2000; **2**: 477-488. [DOI: 10.1186/ar130]; [PMID: 11056678]; [PMCID: PMC17820]
- da Silva Meirelles L, Chagastelles PC, Nardi NB. Mesenchymal stem cells reside in virtually all post-natal organs and tissues. *J Cell Sci*. 2006; **119**: 2204-2213. [DOI: 10.1242/jcs.02932]; [PMID: 16684817]
- Jung Y, Kim JK, Shiozawa Y, Wang J, Mishra A, Joseph J, Berry JE, McGee S, Lee E, Sun H, Wang J, Jin T, Zhang H, Dai J, Krebsbach PH, Keller ET, Pienta KJ, Taichman RS. Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. *Nat Commun*. 2013; **4**: 1795. [DOI: 10.1038/ncomms2766]; [PMID: 23653207]; [PMCID: PMC3649763]
- Ye H, Cheng J, Tang Y, Liu Z, Xu C, Liu Y, Sun Y. Human bone marrow-derived mesenchymal stem cells produced TGFbeta contributes to progression and metastasis of prostate cancer. *Cancer Invest*. 2012; **30**: 513-518. [DOI: 10.3109/07357907.2012.692171]; [PMID: 22646310]
- Rattigan Y, Hsu JM, Mishra PJ, Glod J, Banerjee D. Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu. *Exp Cell Res*. 2010; **316**: 3417-3424. [DOI: 10.1016/j.yexcr.2010.07.002]; [PMID: 20633553]
- Zhang Y, Yang P, Sun T, Li D, Xu X, Rui Y, Li C, Chong M, Ibrahim T, Mercatali L, Amadori D, Lu X, Xie D, Li QJ, Wang XF. miR-126 and miR-126\* repress recruitment of mesenchymal stem cells and inflammatory monocytes to inhibit breast cancer metastasis. *Nat Cell Biol*. 2013; **15**: 284-294. [DOI: 10.1038/ncb2690]; [PMID: 23396050]; [PMCID: PMC3672398]
- Shinagawa K, Kitadai Y, Tanaka M, Sumida T, Kodama M, Higashi Y, Tanaka S, Yasui W, Chayama K. Mesenchymal stem cells enhance growth and metastasis of colon cancer. *Int J Cancer*. 2010; **127**: 2323-2333. [DOI: 10.1002/ijc.25440]; [PMID: 20473928]
- Suzuki K, Sun R, Origuchi M, Kanehira M, Takahata T, Itoh J, Umezawa A, Kijima H, Fukuda S, Saijo Y. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. *Mol Med*. 2011; **17**: 579-587. [DOI: 10.2119/molmed.2010.00157]; [PMID: 21424106]; [PMCID: PMC3146617]
- Kidd SI, Spaeth E, Dembinski JL, Dietrich M, Watson K, Klopp A, Battula VL, Weil M, Andreeff M, Marini FC. Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging. *Stem Cells*. 2009; **27**: 2614-2623. [DOI: 10.1002/stem.187]; [PMID: 19650040]; [PMCID: PMC4160730]
- Jiang J, Chen W, Zhuang R, Song T, Li P. The effect of endostatin mediated by human mesenchymal stem cells on ovarian cancer cells in vitro. *J Cancer Res Clin Oncol*. 2010; **136**: 873-881. [DOI: 10.1007/s00432-009-0728-8]; [PMID: 19921255]
- Choi SA, Lee JY, Wang KC, Phi JH, Song SH, Song J, Kim SK. Human adipose tissue-derived mesenchymal stem cells: characteristics and therapeutic potential as cellular vehicles for prodrug gene therapy against brainstem gliomas. *Eur J Cancer*. 2012; **48**: 129-137. [DOI: 10.1016/j.ejca.2011.04.033]; [PMID: 21664124]
- Lin TM, Chang HW, Wang KH, Kao AP, Chang CC, Wen CH, Lai CS, Lin SD. Isolation and identification of mesenchymal stem cells from human lipoma tissue. *Biochem Biophys Res Commun*. 2007; **361**: 883-889. [DOI: 10.1016/j.bbrc.2007.07.116]; [PMID: 17679141]
- Gibbs CP, Kukekov VG, Reith JD, Tchigrinova O, Suslov ON, Scott EW, Ghivizzani SC, Ignatova TN, Steindler DA. Stem-like cells in bone sarcomas: implications for tumorigenesis. *Neoplasia*. 2005; **7**: 967-976. [DOI: 10.1593/neo.05394]; [PMID: 16331882]; [PMCID: PMC1502023]
- Xu X, Zhang X, Wang S, Qian H, Zhu W, Cao H, Wang M, Chen Y, Xu W. Isolation and comparison of mesenchymal stem-like cells from human gastric cancer and adjacent non-cancerous tissues. *J Cancer Res Clin Oncol*. 2011; **137**: 495-504. [DOI: 10.1007/

- s00432-010-0908-6]; [PMID: 20473524]
24. Cao H, Xu W, Qian H, Zhu W, Yan Y, Zhou H, Zhang X, Xu X, Li J, Chen Z, Xu X. Mesenchymal stem cell-like cells derived from human gastric cancer tissues. *Cancer Lett.* 2009; **274**: 61-71. [DOI: 10.1016/j.canlet.2008.08.036]; [PMID: 18849111]
  25. Xue Z, Wu X, Chen X, Liu Y, Wang X, Wu K, Nie Y, Fan D. Mesenchymal stem cells promote epithelial to mesenchymal transition and metastasis in gastric cancer through paracrine cues and close physical contact. *J Cell Biochem* 2015; **116**: 618-627. [DOI: 10.1002/jcb.25013]; [PMID: 25399738]
  26. Huang F, Wang M, Yang T, Cai J, Zhang Q, Sun Z, Wu X, Zhang X, Zhu W, Qian H, Xu W. Gastric cancer-derived MSC-secreted PDGF-DD promotes gastric cancer progression. *J Cancer Res Clin Oncol.* 2014; **140**: 1835-1848. [DOI: 10.1007/s00432-014-1723-2]; [PMID: 24938433]
  27. Zhu W, Huang L, Li Y, Qian H, Shan X, Yan Y, Mao F, Wu X, Xu WR. Mesenchymal stem cell-secreted soluble signaling molecules potentiate tumor growth. *Cell Cycle.* 2011; **10**: 3198-3207. [DOI: 10.4161/cc.10.18.17638]; [PMID: 21900753]
  28. Zhu W, Huang L, Li Y, Zhang X, Gu J, Yan Y, Xu X, Wang M, Qian H, Xu W. Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo. *Cancer Lett.* 2012; **315**: 28-37. [DOI: 10.1016/j.canlet.2011.10.002]; [PMID: 22055459]
  29. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer* 2009; **9**: 265-273. [DOI: 10.1038/nrc2620]; [PMID: 19262571]
  30. Zhang Q, Wang M, Huang F, Yang T, Cai J, Zhang X, Zhu W, Qian H, Xu W. Pylori infection induced MSC differentiation into CAFs promotes epithelial-mesenchymal transition in gastric epithelial cells. *Int J Mol Med.* 2013; **32**: 1465-1473. [PMID: 24145921]
  31. Kim EK, Kim HJ, Yang YI, Kim JT, Choi MY, Choi CS, Kim KH, Lee JH, Jang WH, Cheong SH. Endogenous gastric-resident mesenchymal stem cells contribute to formation of cancer stroma and progression of gastric cancer. *Korean J Pathol.* 2013; **47**: 507-518. [DOI: 10.4132/KoreanJPathol.2013.47.6.507]; [PMID: 24421843]; [PMCID: PMC3887152]
  32. Guo X, Oshima H, Kitamura T, Taketo MM, Oshima M. Stromal fibroblasts activated by tumor cells promote angiogenesis in mouse gastric cancer. *J Biol Chem.* 2008; **283**: 19864-19871. [DOI: 10.1074/jbc.M800798200]; [PMID: 18495668]
  33. Karnoub AE1, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature.* 2007; **449**: 557-563. [DOI: 10.1038/nature06188]; [PMID: 17914389]
  34. Liu S, Ginstier C, Ou SJ, Clouthier SG, Patel SH, Monville F, Korkaya H, Heath A, Dutcher J, Kleer CG, Jung Y, Dontu G, Taichman R, Wicha MS. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. *Cancer Research.* 2011; **71**: 614-624. [DOI: 10.1158/0008-5472.CAN-10-0538]; [PMID: 21224357]; [PMCID: PMC3100554]
  35. Mishra PJ, Humeniuk R, Medina DJ, Alexe G, Mesirov JP, Ganesan S, Glod JW, Banerjee D. Carcinoma-associated fibroblast-like differentiation of human mesenchymal stem cells. *Cancer Res.* 2008; **68**: 4331-4339. [DOI: 10.1158/0008-5472.CAN-08-0943]; [PMID: 18519693]; [PMCID: PMC2725025]
  36. Quante M, Tu SP, Tomita H, Gonda T, Wang SS, Takashi S, Baik GH, Shibata W, Diprete B, Betz KS, Friedman R, Varro A, Tycko B, Wang TC. Bone marrow-derived myofibroblasts contribute to the mesenchymal stem cell niche and promote tumor growth. *Cancer Cell.* 2011; **19**: 257-272. [DOI: 10.1016/j.ccr.2011.01.020]; [PMID: 21316604]; [PMCID: PMC3060401]
  37. Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. *Science.* 2004; **306**: 1568-1571. [DOI: 10.1126/science.1099513]; [PMID: 15567866]
  38. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, Betz KS, Penz-Oesterreicher M, Bjorkdahl O, Fox JG, Wang TC. Overexpression of interleukin-1 beta induces gastric inflammation and cancer and mobilize myeloid-derived suppressor cells in mice. *Cancer Cell.* 2008; **14**: 408-419. [DOI: 10.1016/j.ccr.2008.10.011]; [DOI: 10.1016/j.ccr.2008.11.004]; [PMID: 18977329]; [PMCID: PMC2586894]
  39. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, Carey VJ, Richardson AL, Weinberg RA. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL 12 secretion. *Cell.* 2005; **121**: 335-348. [DOI: 10.1016/j.cell.2005.02.034]; [PMID: 15882617]
  40. Stappenbeck TS, Miyoshi H. The role of stromal stem cells in tissue regeneration and wound repair. *Science.* 2009; **324**: 1666-1669. [DOI: 10.1126/science.1172687]; [PMID: 19556498]
  41. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature.* 2007; **449**: 557-563. [DOI: 10.1038/nature06188]; [PMID: 17914389]
  42. Kabashima-Niibe A, Higuchi H, Takaishi H, Masugi Y, Matsuzaki Y, Mabuchi Y, Funakoshi S, Adachi M, Hamamoto Y, Kawachi S, Aiura K, Kitagawa Y, Sakamoto M, Hibi T. Mesenchymal stem cells regulate epithelial-mesenchymal transition and tumor progression of pancreatic cancer cells. *Cancer Sci.* 2012; **104**: 157-164. [DOI: 10.1111/cas.12059]; [PMID: 23121112]
  43. Kalluri R, Zeisberg M. Fibroblasts in cancer. *Nat Rev Cancer.* 2006; **6**: 392-401. [DOI: 10.1038/nrc1877]; [PMID: 16572188]
  44. Giannoni E, Bianchini F, Masieri L, Serni S, Torre E, Calorini L, Chiarugi P. Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. *Cancer Res.* 2010; **70**: 6945-6956. [DOI: 10.1158/0008-5472.CAN-10-0785]; [PMID: 20699369]
  45. Worthley DL, Ruzsiewicz A, Davies R, Moore S, Nivison-Smith I, Bik To L, Browett P, Western R, Durrant S, So J, Young GP, Mullighan CG, Barty PG, Michael MZ. Human gastrointestinal neoplasia-associated myofibroblasts can develop from bone marrow-derived cells following allogenic stem cell transplantation. *Stem Cells.* 2009; **27**: 1463-1468. [DOI: 10.1002/stem.63]; [PMID: 19492298]
  46. Klymkowsky MW, Savagner P. Epithelial-mesenchymal transition: a cancer researcher's conceptual friend and foe. *Am J Pathol* 2009; **174**: 1588-1593. [DOI: 10.2353/ajpath.2009.080545]; [PMID: 19342369]; [PMCID: PMC2671246]
  47. Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits. *Nat Rev Cancer.* 2009; **9**: 265-273. [DOI: 10.1038/nrc2620]; [PMID: 19262571]
  48. Zhang Q, Wang M, Huang F, Yang T, Cai J, Zhang X, Zhu W, Qian H, Xu W. Pylori infection-induced MSC differentiation into CAFs promotes epithelial-mesenchymal transition in gastric epithelial cells. *Int J Mol Med.* 2013; **32**: 1465-1473. [PMID: 24145921]
  49. Wang J, Yu J, Wu J, Wang S, Chen D, Yang F, Hua B, Xi X, Song S, Zheng L, Jiang B. Experimental study of human bone marrow mesenchymal stem cells on regulating the biological characteristics of gastric cancer cells. *Zhonghua Wei Chang Wai Ke Za Zhi.* 2015; **18**: 159-165. [PMID: 25656126]
  50. Zhu W, Huang L, Li Y, Qian H, Shan X, Yan Y, Mao F, Wu X, Xu WR. Mesenchymal stem cell secreted soluble signaling molecules potentiate tumor growth. *Cell Cycle.* 2011; **10**: 3198-3207. [DOI: 10.4161/cc.10.18.17638]; [PMID: 21900753]
  51. Huang F, Wang M, Yang T, Cai J, Zhang Q, Sun Z, Wu X, Zhang X, Zhu W, Qian H, Xu W. Gastric cancer-derived MSC-secreted

- PDGF-DD promotes gastric cancer progression. *J Cancer Res Clin Oncol.* 2014; **140**: 1835-1848. [DOI: 10.1007/s00432-014-1723-2]; [PMID: 24938433]
52. Kuo CH, Liu CJ, Lu CY, Hu HM, Kuo FC, Liou YS, Yang YC, Hsieh MC, Lee OK, Wu DC, Wang SS, Chen YL. 17 $\beta$ -estradiol inhibits mesenchymal stem cells-induced human AGS gastric cancer cell mobility via suppression of CCL5- Src/Cas/Paxillin signaling pathway. *Int J Med Sci.* 2013; **11**: 7-16. [DOI: 10.7150/ijms.6851]; [PMID: 24396281]; [PMCID: PMC3880986]
  53. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. *Oncogene.* 2004; **23**: 7274-7282. [DOI: 10.1038/sj.onc.1207947]; [PMID: 15378087]
  54. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumor initiating cells. *Nature.* 2004; **432**: 396-401. [DOI: 10.1038/nature03128]; [PMID: 15549107]
  55. Eramo A, Lotti F, Sette G, Pilozi E, Biffoni M, Di Virgilio A, Conticello C, Ruco L, Peschle C, De Maria R. Identification and expansion of the tumorigenic lung cancer stem cell population. *Cell Death Differ.* 2008; **15**: 504-514. [DOI: 10.1038/sj.cdd.4402283]; [PMID: 18049477]
  56. Ma S, Chan KW, Hu L, Lee TK, Wo JY, Ng IO, Zheng BJ, Guan XY. Identification and characterization of tumorigenic liver cancer stem/progenitor cells. *Gastroenterology* 2007; **132**: 2542-2556. [DOI: 10.1053/j.gastro.2007.04.025]; [PMID: 17570225]
  57. O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumor growth in immunodeficient mice. *Nature.* 2007; **445**: 106-110. [DOI: 10.1038/nature05372]; [PMID: 17122772]
  58. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C. Identification and expansion of human colon cancer-initiating cells. *Nature.* 2007; **445**: 111-115. [DOI: 10.1038/nature05384]; [PMID: 17122771]
  59. Hermann PC, Huber SL, Herrler T, Aicher A, Ellwart JW, Guba M. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. *Cell Stem Cell.* 2007; **1**: 313-323. [DOI: 10.1016/j.stem.2007.06.002]; [PMID: 18371365]
  60. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. *Cancer Res.* 2005; **65**: 10946-10951. [DOI: 10.1158/0008-5472.CAN-05-2018]; [PMID: 16322242]
  61. Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, Hung SC, Chang YL, Tsai ML, Lee YY, Ku HH, Chiou SH. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. *PLoS One.* 2008; **3**: e2637. [DOI: 10.1371/journal.pone.0002637]; [PMID: 18612434]; [PMCID: PMC2440807]
  62. Zhao P, Lu Y, Jiang X, Li X. Clinicopathological significance and prognostic value of CD133 expression in triple-negative breast carcinoma. *Cancer Sci.* 2011; **102**: 1107-1111. [DOI: 10.1111/j.1349-7006.2011.01894.x]; [PMID: 21276138]
  63. Hagiwara S, Kudo M, Ueshima K, Chung H, Yamaguchi M, Takita M, Haji S, Kimura M, Arao T, Nishio K, Park AM, Munakata H. The cancer stem cell marker CD133 is a predictor of the effectiveness of S1+pegylated interferon  $\alpha$ -2b therapy against advanced hepatocellular carcinoma. *J Gastroenterol.* 2011; **46**: 212-221. [DOI: 10.1007/s00535-010-0294-5]; [PMID: 20683621]
  64. Ong CW, Kim LG, Kong HH, Low LY, Iacopetta B, Soong R, Salto-Tellez M. CD133 expression predicts for non-response to chemotherapy in colorectal cancer. *Mod Pathol* 2010; **23**: 450-457. [DOI: 10.1038/modpathol.2009.181]; [PMID: 20081809]
  65. Hayashi T1, Ding Q, Kuwahata T, Maeda K, Miyazaki Y, Matsubara S, Obara T, Natsugoe S, Takao S. Interferon-alpha modulates the chemosensitivity of CD133-expressing pancreatic cancer cells to gemcitabine. *Cancer Sci.* 2012; **103**: 889-896. [DOI: 10.1111/j.1349-7006.2012.02235.x]; [PMID: 22320450]
  66. Hashimoto K, Aoyagi K, Isobe T, Kouhujii K, Shirouzu K. Expression of CD133 in the cytoplasm is associated with cancer progression and poor prognosis in gastric cancer. *Gastric Cancer.* 2014; **17**: 97-106. [DOI: 10.1007/s10120-013-0255-9]; [PMID: 23558457]; [PMCID: PMC3889295]
  67. Semba S. Bone marrow-derived mesenchymal stem cells provide an advantageous tumor microenvironment for the restoration of gastric cancer stem cells. *Kenbikyō* 2011; **46**: 89-94.
  68. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. *Annu Rev Cell Dev Biol.* 1998; **14**: 59-88. [DOI: 10.1146/annurev.cellbio.14.1.59]; [PMID: 9891778]
  69. Wong GT, Gavin BJ, McMahon AP. Differential transformation of mammary epithelial cells by wnt genes. *Mol Cell Biol.* 1994; **14**: 6278-6286. [DOI: 10.1128/MCB.14.9.6278]; [PMID: 8065359]; [PMCID: PMC359154]
  70. Kuhl M, Sheldahl LC, Park M, Miller JR, Moon RT. The Wnt/Ca<sup>2+</sup> pathway: a new vertebrate wnt signaling pathway takes shape. *Trends Genet* 2000; **16**: 279-283. [DOI: 10.1016/S0168-9525(00)02028-X]
  71. Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3 $\beta$  and b-catenin and promotes GSK-3 $\beta$ -dependent phosphorylation of b-catenin. *EMBO J.* 1998; **17**: 1371-1384. [DOI: 10.1093/emboj/17.5.1371]; [PMID: 9482734]; [PMCID: PMC1170485]
  72. Kitagawa M, Hatakeyama S, Shirane M, Matsumoto M, Ishida N, Hattori K, Nakamichi I, Kikuchi A, Nakayama K, Nakayama K. An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of b-catenin. *EMBO J.* 1999; **18**: 2401-2410. [DOI: 10.1093/emboj/18.9.2401]; [PMID: 10228155]; [PMCID: PMC1171323]
  73. Kikuchi A. Roles of Axin in the Wnt signaling pathway. *Cell Signal.* 1999; **11**: 777-788. [DOI: 10.1016/S0898-6568(99)00054-6]
  74. Liu C, Li Y, Semenov M, Han C, Baeq GH, Tan Y, Zhang ZH, Lin XH, He X. Control of b-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell.* 2002; **108**: 837-847. [DOI: 10.1016/S0092-8674(02)00685-2]
  75. He X, Semenov M, Tamai K, Zeng X. LDL receptor-related proteins 5 and 6 in Wnt/b-catenin signaling: arrows point the way. *Development* 2004; **131**: 1663-1677. [DOI: 10.1242/dev.01117]; [PMID: 15084453]
  76. Hurlstone A, Clevers H. T-cell factors: turn-ons and turn-offs. *EMBO J.* 2002; **21**: 2303-2311. [DOI: 10.1093/emboj/21.10.2303]; [PMID: 12006483]; [PMCID: PMC126013]
  77. Kohn AD, Moon RT. Wnt and calcium signaling: b-catenin-independent pathways. *Cell Calcium.* 2005; **38**: 439-446. [DOI: 10.1016/j.ceca.2005.06.022]; [PMID: 16099039]
  78. Topol N, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent b-catenin degradation. *J Cell Biol.* 2003; **162**: 899-908. [DOI: 10.1083/jcb.200303158]; [PMID: 12952940]; [PMCID: PMC2172823]
  79. Ishitani T, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, Shibuya H, Moon RT, Ninomiya-Tsuji J, Matsumoto K. The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt-5a/Ca<sup>2+</sup> pathway to antagonize Wnt/b-catenin signaling. *Mol Cell Biol.* 2003; **23**: 131-139. [DOI: 10.1128/MCB.23.1.131-139.2003]; [PMID: 12482967]; [PMCID: PMC140665]
  80. Mikels AJ, Nusse R. Purified Wnt5a protein activates or inhibits b-catenin-TCF signaling depending on receptor context. *PLoS Biol.* 2006; **4**: 570-582. [DOI: 10.1371/journal.pbio.0040115]; [PMID: 16602827]; [PMCID: PMC1420652]
  81. Iozzo RV, Eichstetter I, Danielson KG. Aberrant expression of the growth factor Wnt-5A in human malignancy. *Cancer Res.* 1995; **55**: 3495-3499. [PMID: 7627953]
  82. Weeraratna AT1, Jiang Y, Hostetter G, Rosenblatt K, Duray P, Bittner M, Trent JM. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell.* 2002; **1**: 279-

288. [DOI: 10.1016/S1535-6108(02)00045-4]; [PMID: 12086864]
83. Pukrop T, Klemm F, Hagemann T, Gradl D, Schulz M, Siemes S, Trümper L, Binder C. Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA*. 2006; **103**: 5454-5459. [DOI: 10.1073/pnas.0509703103]; [PMID: 16569699]; [PMCID: PMC1459376]
84. Kurayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, Asahara T, Yasui W, Kikuchi A. Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. *Cancer Res*. 2006; **66**: 10439-10448. [DOI: 10.1158/0008-5472.CAN-06-2359]; [PMID: 17079465]
85. Bruna A, Darken RS, Rojo F, Ocaña A, Peñuelas S, Arias A, Paris R, Tortosa A, Mora J, Baselga J, Seoane J. High TGFbeta-Smad activity confers poor prognosis in glioma patients and promote cell proliferation depending on the methylation of the PDGF-B gene. *Cancer Cell*. 2007; **11**: 147-160. [DOI: 10.1016/j.ccr.2006.11.023]; [PMID: 17292826]
86. Matsuyama S, Iwadate M, Kondo M, Saitoh M, Hanyu A, Shimizu K, Aburatani H, Mishima HK, Imamura T, Miyazono K, Miyazawa K. SB-431542 and Gleevec inhibit transforming growth factor-beta-induced proliferation of human osteosarcoma cells. *Cancer Res*. 2003; **63**: 7791-7798. [PMID: 14633705]
87. Derynck R, Zhang YE. Smad -dependent and Smad-independent pathways in TGF-beta family signaling. *Nature*. 2003; **425**: 577-584. [DOI: 10.1038/nature02006]; [PMID: 14534577]
88. Massagué J. TGFbeta in cancer. *Cell*. 2008; **134**: 215-230. [DOI: 10.1016/j.cell.2008.07.001]; [PMID: 18662538]; [PMCID: PMC3512574]
89. Miyazawa K, Shinozaki M, Hara T, Furuya T, Miyazono K. Two major pathways in TGF-beta superfamily signaling. *Genes Cells*. 2002; **7**: 1191-1204. [DOI: 10.1046/j.1365-2443.2002.00599.x]; [PMID: 12485160]
90. Golenstaneh N, Mishra B. TGF-beta, neuronal stem cells glioblastoma. *Oncogene*. 2005; **24**: 5722-5730. [DOI: 10.1038/sj.onc.1208925]; [PMID: 16123805]
91. Ikushima H, Todo T, Ino Y, Takahashi M, Miyazawa K, Miyazono K. Autocrine TGF- $\beta$  signaling maintains tumorigenicity of glioma-initiating cells through sry-related HMG-box factors. *Cell Stem Cell*. 2009; **5**: 504-514. [DOI: 10.1016/j.stem.2009.11.011]; [DOI: 10.1016/j.stem.2009.08.018]; [PMID: 19896441]

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