The Identifications and Clinical Implications of Cancer Stem Cells in Colorectal Cancer

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Abstract

Cancer stem cells (CSCs) are cancer cells that are responsible for initiation, progression, metastasis, and recurrence in cancer. The aim of this review was to analyze the markers for identifying of CSCs in colorectal carcinoma, as well as the prognostic and therapeutic implications of these markers in the cancer. CSCs are insensitive to the current drug regimens. In colorectal carcinoma, markers, including Nanog, Oct-4, SOX-2, Lgr-5, CD133, CD24, CD29, ALDH1, EpCAM, CD44, CD166, and CD26, are commonly used for the identification and isolation of CSCs. In addition, ALDH1, CD24, CD44, CD133, CD166, EpCAM, Lgr-5, Nanog, and SOX-2 could have clinical roles in predicting pathological stages, cancer recurrence, therapy resistance, and patients' survival in patients with colorectal carcinoma. In light of the current knowledge of CSCs in colorectal carcinoma, novel potential therapeutic strategies, such as development of monoclonal antibodies or immunotoxins and targeting various cell surface molecules in colorectal CSCs and/or components of signaling pathways, have been developed. This could open new opportunities for the better management of patients with colorectal carcinoma.

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Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in men and the second in women. In 2012, there were an estimated 1.4 million incidences and 693,900 deaths throughout the world.[1] Failure of treatment of patients with CRC could be attributed to the escaped residual microscopic carcinoma after surgery, which later initiates the metastatic process.[2] In principle, these residual cancer cells are eliminated by postoperative chemotherapy and/or radiotherapy. However, the presence of therapy-resistant cancer cells limits the success of these treatments.[3,4] Genetic, epigenetic, and functional heterogeneity of cancer cells supports the existence of these therapy-resistant cancer cells in patients with CRC.[5-7] These small fractions of cells within the cancers are called cancer stem cells (CSCs). These CSCs are capable of initiating, maintaining, and developing cancer growth.[3,8] Also, CSCs have self-renewal capacity and are responsible for developing functionally and morphologically diverse cells, including therapy-resistant and metastatic cell populations.[3]

CSCs have been implicated in colorectal carcinogenesis for a long time, although their existence has been only recently demonstrated experimentally.[9,10] In view of the importance of CSCs in CRC, we aimed to review the markers for identifying of CSCs in CRC as well as the prognostic and therapeutic implications of these markers in CRC.

Identification of CSCs

By definition, CSCs are the cells that have the capacity to drive carcinogenesis through long-term production and self-renewal of differentiated, nontumorigenic progenies.[11] It was also reported that chemoradiotherapy-resistant CSCs have greater potential of tumor initiation and stimulated the regrowth of cancer after a therapeutic treatment.[12-15] The existence and the identity of CSCs have been reported for the first time in hematopoietic cancers.[16] Thereafter, CSCs from many solid cancers, such as those arising from breast, brain, prostate, head, and neck, were also identified.[12,13,17]

The current gold standard for defining CSC "stemness" is to show their ability to transfer disease into immunodeficient mice at a limited dilution.[14,15] This type of xenograft assay involves fluorescence-activated cell sorting of a single cancer cell that has the putative CSC properties and demonstrates its ability to develop a new cancer similar to the original cancer.[14,15,18] The limitation of this method is partly related to the difficulties discriminating

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between CSC and non-CSC populations of cancer cells. Also, the difference between the microenvironment of the original cancer and the transplanted recipient may have an impact on the function of CSCs.[19] Thus, the identification and isolation of a CSC is still a matter of debate due to lack of unique methods for isolation and identification as well as their complex biology.[9,10]

Identification of CSCs in CRC

Genes such as *Nanog*, *Oct-4*, and *SOX-2* are responsible for the pluripotency of cells and are commonly considered to be the surrogate markers for CSCs (Table 1).[20,21]

Nanog, a homeobox protein encoded by *Nanog*, is a transcription factor and regulates the stem cell properties, especially the self-renewal pluripotency of cells.[22] Matsuoka and colleagues[23] showed that nanog was positive in 28 (10%) of 290 gastric cancer tissues. In CRC, Meng and colleagues[24] highlighted the importance of Nanog in the maintenance of cell proliferation, invasion, and motility of CRC cells as well as its contribution to the epithelial mesenchymal transition (EMA) in the development of CRC.

Oct 4 (a member of the POU family) contributes to the self-renewal ability and inhibits the genes responsible for differentiation as well as to enable the self-renewal ability of stem cells.[25,26] Padín-Iruegas and colleagues[27] demonstrated that Oct4 mRNA was present in the peripheral blood of patients with metastatic CRC.

Sex-determining region Y (SRY)-box 2 (SOX-2) is a stem cell marker and plays crucial roles in the maintenance of cell pluripotency and self-renewal.[28-30] In addition, it has been reported that SOX-2 plays an important role in the maintenance of self-renewal of CSCs.[31] Knockdown of *SOX-2* and *Oct4* reduced the tumor size in oral cancer in immunodeficient mice.[32] Furthermore, SOX-2 was found positive in 159 (55%) of 290 gastric cancers.[23] In CRC, SOX-2 has been used to identify the CSCs in many studies.[33-35]

O'Brien and his group[15] noted that CD133-positive human cancer cells were able to produce cancer of similar morphology to the original one in immunodeficient mice, whereas the CD133-negative cells were unable to initiate cancer growth. CD133 has been used to study 501 CRC on tissue microarrays in CRC.[34]

Leucine-rich repeat-containing G-protein–coupled receptor 5 (Lgr-5)–positive cells (Lgr-5+) have the characteristic features of CSCs in CRC.[36-40] Schepers and colleagues[38] demonstrated that some cells within the mouse colonic adenoma (5%-10%) were Lgr-5+ cells. These cells were responsible for self-renewal and production of differentiated Lgr-5− colonic adenoma cells. It was reported that patients with CRC expressing high Lgr-5 had 10-fold higher risk for cancer relapse than patients with low expression of Lgr-5.[39] In addition, it has been demonstrated that Lgr-5+ cells derived from patients with CRC have the potential of CSC as they showed high number of spheroid formation in culture conditions.[41] Therefore, Lgr-5 has the potential to be used as a surrogate marker for the identification of CSCs in CRC.

Cluster of differentiation 24 (CD 24), also called heat stable antigen 24 (HAS) or signal transducer 24, is a glycoprotein and expressed at the cell surface of lymphocytes.[42] Rowehl and colleagues[43] reported the establishments of CRC's CSCs using in vitro and in vivo mouse models from liver metastasis of patients with colon cancer. This study also demonstrated that CD24+ cells were highly tumorigenic and clonogenic with increased stemness and pluripotency and exhibited resistance to therapy.[43] Sahlberg and colleagues[44] reported that colon cancer cells expressing CD24, CD133, and CD44 act as CSCs and was associated with radiation resistance in colon cancer cells. Thus, CD24 can be used as a putative marker for CSC isolation and identification in CRCs.

CD29, also called integrin beta-1 protein, is encoded by the *ITGB1* gene. It plays a key role in cell adhesion and various cellular processes like embryogenesis, hemostasis, tissue repair, immune response, and cancer metastases.[45] CD29 is reported to be a surface marker for the highly proliferative site of human colonic crypt, and thereby CD29-positive cells could be used as a marker for stem cell type in human colon.[46] In addition, high expressions of CD29 were noted in human colon CSCs and these cells acted as tumor initiator/CSCs in mouse colonic carcinoma.[47] Another study has identified that colon CSCs with phenotypic fractions of CD^{29+}/CD^{133+}cells exhibited distinct proliferation, differentiation, and self-renewal properties.[48] These studies suggest that CD29 can be used as a surface marker in identifying CSCs in colon cancers.

Aldehyde dehydrogenase isoform 1 (ALDH1) is an isoform of aldehyde dehydrogenase enzyme and catalyses the conversion of aldehyde to carboxylic acid.[49] This enzyme is commonly used as a surrogate marker for the identification of non-CSCs as well as CSCs in different cancers, including breast cancer, pancreatic cancer,

Table 1 Biomarkers of Colorectal Cancer Stem Cells

Protein Markers	Gene	Assay Method	References
Nanog, Oct-4, SOX-2	*Nanog*, *POU5F1*, *SOX-2*	Therapy-resistant assay; quantitative reverse transcriptase polymerase chain reaction	20-24,33
CD133	*PROM1*	Chemoresistance assay; colony formation assay	9,15,34,98-100
Lgr-5	*LGR5*	Tumorigenicity assay; experimental metastasis assay	40,47
CD24	*CD24*	Colony formation assay; invasion assay; differentiation assay; survival assay	47,84,85
CD29	*ITGB1*	Colony formation assay	47
ALDH-1	*ALDH1A1*	Xenotransplantation in immunodeficient mice	51,72
EpCAM	*EPCAM*	Immunohistochemistry; Western blot assay	58,98
CD44	*CD44*	Xenotransplantation in immunodeficient mice; colony formation assay	47,58,88,136
CD166	*ALCAM*	Tumor growth in immunodeficient mice following xenograft; colony formation assay	47
CD26	*DPP4*	Tumor formation and metastasis following xenotransplantation	69