Cancer stem cells: An overview of the pathophysiological and prognostic roles in colorectal cancer

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Abstract

Colorectal cancer (CRC) is one of the most common types of cancer globally. A subpopulation of tumour cells, cancer stem cells (CSC), is speculated to be responsible for tumor initiation, development, maintenance, metastasis, and recurrence. Colorectal cancer stem cells (CRC-SCs) have demonstrated some unique features, including a high level of heterogeneity and plasticity. This review summarizes the current understanding of CRC-SCs in the progression of CRC and the prognostic importance of these CSCs and their markers in colorectal cancer.

Keywords: CRC-SC, signal transduction, therapeutic approaches

Introduction

Colorectal cancer (CRC) is the third most common type of malignancy globally (1). In colorectal carcinogenesis, several processes are involved, including genetic and epigenetic errors (2), microenvironmental crosstalk (3), cell of origin, and cell type (4). Under the origin and type of cells, two models for colorectal carcinogenesis (cancer stem cell (CSC) model and stochastic model) have been reported (5). In the stochastic model, as a traditional model, any cell within the tumor population can develop cancer in the initiation and progression stages. However, the CSC model suggests that CSCs with lower cells are capable of tumor progression and metastasis (5).

Regulation of the different conditions of stem cells (SCs) in a functional unit of the intestinal crypt is dependent on the reciprocal relationship between them and the intestinal microenvironment (niche) (6). As cellular and extracellular elements, the niche regulates the ideal conditions of SCs and asymmetrical division of these cells into two different daughter cells via secretion of the various growth factors, cytokines, and chemokines (7). Furthermore, in the intestinal subepithelial, myofibroblast cells also adjust differentiation and self-renewal of SCs by mediating crosstalk between epithelial and mesenchymal cells and secretion of morphogenic factors (8). Finally, the epithelial-mesenchymal interactions control the balance between proliferation, differentiation, migration, and renewal of both the divided and asymmetric division of SCs by involving several signal transductions in these cells (8). However, disorders of genetic contents of niche compartments lead to the abnormal condition of SCs and contrariwise (9). The niche aberrations consist of transformed myofibroblasts, myeloid cells, and extracellular agents that accelerate dedifferentiation and nonrenewal of SCs, as well as the symmetric

division of SCs via secretion of various cytokines, chemokines, and growth factors including hepatocyte growth factor (HGF), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) (8, 9). According to such conditions, colorectal cancer stem cells (CRC-SCs) are initiated, stimulating carcinogenesis, invasiveness, and metastasis through over-expression of tumor genes mediated by aberrant activation of several signal transductions (10).

CRC-SCs typically comprise of a small fraction of the tumor-derived cells population (5). These cells are considered the source of stemness, recurrence, chemoresistance, and tumorigenesis in colorectal cancer-derived tumors (11). Examining the SCs and CRC-SCs properties is complex due to the absence of unique molecular markers and techniques. For identification and isolation of both SCs and CRC-SCs, only assumptions have been established regarding their precise number, position, and specific properties (12). Several factors have been proposed as targets of CRC-SCs, including the cluster of differentiation (CD)-24, CD44, CD133, CD166, CD29, CD26, Nanog, Oct-4, SOX-2, leucine-rich repeat containing G protein-coupled receptor (Lgr-5), aldehyde dehydrogenase-1 (ALDH-1), and EpCAM. These markers could also have various key clinical functions in progression, metastasis, stages of the disease, and resistance to distinct therapeutic methods. Among the probable markers for CRC-SCs, some markers targets SCs such as Musashi-1 (Msi-1), Bmi-1, Lgr-5, ALDH-1, CD29, Tert, and achaete scute-like 2 (Ascl-2) during homeostasis. This review summarizes the importance of CSCs in the progression of colorectal tumors and the prognostic value of CSCs markers in CRC.

Signal transductions involved in the intestinal/colon SCs and CRC-SCs

In humans, during stable conditions between niche and SCs, the intestinal homeostasis is established by the highest turnover rate every 5 to 7 days (1). This rapid renewal process is by asymmetric division and proliferation of SCs at intestinal crypts base, migration, and differentiation of SCs at intestinal villus. This is mediated by several signal transduction processes, including Wingless/Int (Wnt), Hedgehog, Notch, platelet-derived growth factor, and bone morphogenic protein (BMP) (8). The aberrant activation of signaling pathways caused by genetic contents of SCs and niche lead to accruing of resistance colorectal cancer stem cells (CCSCs) against chemotherapy and radiotherapy during the treatment of patients with CRC (13, 14).

Wnt signaling pathway

The Wnt signaling pathway is divided into two distinct pathways: canonical or Wnt β-catenin dependent and non-canonical or Wnt β-catenin independent pathways (15-17). The canonical Wnt signaling plays a critical role in cell proliferation and differentiation processes (16). It has been demonstrated that the canonical Wnt signaling is aberrantly activated in CRC (18). The non-canonical Wnt signaling is composed of two signaling pathways, namely Wnt calcium (Wnt/Ca^{2+}) and Wnt planar cell polarity (Wnt/PCP) pathways (19). They regulate cell adhesion, tissue separation, migration, cytoskeleton rearrangements, and actin polymerization (20-22).

The Wnt signaling has been detected at the intestinal crypt base region, which regulates rapid regeneration of the intestinal homeostasis through cell proliferation of SCs (23). In intestinal homeostasis, the niche compartments stimulate SCs cell proliferation, resulting in an asymmetric division of these cells through moderate activation of Wnt signaling-related genes, including cyclin D and c-myc (24). In line with this, the Wnt signaling plays

a key role in maintaining intestinal homeostasis, development, and proliferation (18). Under alterations of niche, transformed cells at the crypt promote Wnt signaling-Induced CSCs cell proliferation via releasing of chemokines, cytokines, and growth factors including HGF, IL-6, and TNF-α (9).

It has been demonstrated that transformed fibroblasts provoke Wnt-induced cell proliferation of CSCs by over-expression of HGF (9). HGF is probably known as an enhancer Wnt activity which can also be affected β-catenin activity, as a critical transcriptional regulator, in CSCs stemness (9). Like HGF, TNF-α is another cytokine that leads to the development of colorectal cancer through over-activation of Wnt/β-catenin signaling (9). In addition, one study reported that TNF-α-induced downregulation of Caudal-related homeobox transcription factor 2 (CDK2) also stimulates over activation of Wnt-mediated unusual cell proliferation by low-expression of adenomatous polyposis coli (APC), axis inhibition protein 2 (AXIN2), glycogen synthase kinase-3 beta (GSK3β), and over-expression of Wnt target genes (25).

Wnt signaling-related target genes including *cyclin D1*, *c-Myc*, and *APC* are involved in different cellular processes of intestinal CSCs (26). As a key tumour suppressor, the aberrant genetic alterations in SCs such as APC result in tumorigenic niche and subsequently activate Wnt signaling-induced cell proliferation of CSCs (26). In a study, doxycycline-regulated short hairpin RNA (shRNA)-induced conditional silencing of *APC* promoted adenomas by stimulating both Wnt target genes, including *Axin2* and *c-Myc*, as well as ISCs markers, such as Lgr5, Ascl2, and Sox9 (26). The findings of one study showed that renewal APC operated normal balance between proliferation and differentiation, and eventually the intestinal homeostasis, even in the presence of *Kras*

and *p53* mutations (26). Deleting APC in Lrg5^{+} colonic CSCs activated by Wnt leads to the transformation of microadenoma into macroadenoma at the crypt base. This suggests that over-activated Wnt signaling caused by *APC* mutation may trigger the development of tumor progression (27). Following *APC* mutation in intestinal SCs and colonic CSCs, cell proliferation and tumorigenesis are established by stable Wnt signaling activity-related target genes, including *cyclin D1* and *c-Myc* (28). The expression levels of c-Myc, as a proto-oncogene, regulates the balance between proliferation and differentiation through p21 promoter transcription-mediated G1 arrest in both SCs and CSCs (29). Upregulation of *c-Myc* levels caused by APC loss significantly impacts all phenotypes and tumorigenesis through over-activation of Wnt signaling in APC-deficient intestinal epithelium (30). Over-expression of c-Myc influences tumor progression process via strong bind to an enhancer region in *TCF7L2* gene named rs6983267 polymorphism. Finally, over-activation of Wnt signaling is associated with an increased risk of colorectal cancer in intestinal CSCs (30). These results supported that high expression of c-Myc alone cannot stimulate tumorigenesis and alter phenotypes of APC loss in APC-deficient cells.

The Wnt signaling pathway also indirectly increase converting non-CSCs into CSCs, invasiveness, and metastasis of CSCs by provoking epithelial-mesenchymal transition (EMT) in the primary tumor (31, 32).

Notch signaling pathway

Notch ligands are typical and atypical proteins which, upon binding the family Notch receptors, including Notch1, Notch2, Notch3 or Notch4, results in the release of γ-secretase-induced notch intracellular domain (NICD). This eventually leads to the

activation of two intracellular signaling pathways, namely canonical and non-canonical pathways. Activation of canonical Notch pathway by over-expression of downstream target genes *HES*1 and *HEY*1 lead to suppression of differentiation and maintenance of CSCs of stemness (33).

In intestinal homeostasis, activation of several signal transductions, particularly notch signaling in epithelial and mesenchymal cells, mediate the balance between proliferation and differentiation of divided SCs (8). To alter the genetic contents of niche and SCs leads to increased gene expression of the *notch* in stromal cells and SCs. Subject to conditions, aberrant activation of the notch signaling pathway can finally influence the cancer-initiating stemness of CRC-SCs, metastasis, tumor growth, and CRC progression (8). In one study, Notch1 promotes CRC-CSs-induced colonosphere formation via abnormal activation of notch signaling while markers of colonosphere and CRC-SCs including $CD133^{+}$, $CD44^{+}$, and $EPCAM^{+}$ was maintained (34). Following the formation of the colonosphere, notch1 can potentially stimulate tumor growth and eventually metastasis through the activation of notch signaling (34). It has also been reported that inhibition of notch signaling caused by N- [N- (3,5-difluorophacetyl)-l-alanyl]-S-phenylglycine t-butyl ester (DAPT), as a γ-secretase inhibitor, reduces the number of CRC-SC colonosphere and expression levels of notch1 in HCT116 cell lines-derived CRC-SCs (34). In another supporting role of notch signalling in CRC-SCs, Meng and colleagues showed enhanced CRC cells' chemosensitivity by suppressing Notch signaling cascade with γ-secretase inhibitors (GSI). They demonstrated that both the Notch1 receptor and *HES1* and chemoresistance target genes are highly expressed in colon cancer cells associated with CRC progression (35). There was a positive correlation between HES1 expression and

stem cell markers expression, mediated by *HES1*-induced increasing the $CD133^+$ cells sizes (36). Sikandar et al. investigated the role of Notch signaling in the self-renewal of colon cancer-initiating cells (CRC-IC) by short hairpin RNA knockdown and small-molecule inhibition. They reported that the Notch signaling is 10- to 30- fold higher in CRC-IC compared with colon cancer cell lines. Findings of the study demonstrated that Notch signaling inhibits apoptosis via suppression of both cell cycle kinase inhibitor *P27* and transcription factor *ATOH1* in CRC-IC (37). Several studies showed that various types of signaling pathways, such as Notch signaling, could lead to the proliferation of CSCs in CRC-SCs, and dysregulation of Notch signaling stimulates the development of CRC (38). As a tumour suppressor, switching the targets of Notch signaling such as notch1 induced by microRNA (miR)-34a can lead to the selection of between self-renewal and differentiation in CRC-SCs (39).

Bone morphogenic protein (BMP) signaling pathway

BMPs, as ligands of transforming growth factor β (TGF-β) superfamily, activate BMP signaling via phosphorylation of receptors of type 1 and type 2 serine-threonine kinases (40). The activated type 1 receptor phosphorylates receptor-associated SMADs (SMAD1,5,8), which later bind to SMAD4 and translocate into the nucleus to control the expression of genes involved in cellular processes (41).

Further signal transduction as a key regulator of intestinal homeostasis and balance between cellular processes is a bone morphogenic protein (BMP) (42). The BMP signaling compartments are expressed in stromal cells, and BMP antagonists are mainly expressed in mesenchymal cells (42). This pathway is mainly activated in the epithelial and mesenchymal cells at the top of the crypt. It regulates the differentiation and

apoptosis of divided SCs, differentiated cells, and epithelial and mesenchymal cells (43). In the intestinal homeostasis state, the BMP signalplaysplay the main function in differentiation and balance between differentiation and proliferation of divided SCs (44). To change genetic stores of niche including mutations of BMP signaling elements cause tumor progression. In further support of the role of BMP mutations in tumorigenesis, it has been reported that BMP-deficient stromal cells may result in adenoma formation by aberrant activation of Wnt/β-catenin and inactivation of BMP signaling (45).

BMP4, as an inducer of the differentiation stage, has low and high expression in tumor-isolated CRC-SCs and differentiated cells, respectively (46). Hence, administration of recombinant BMP4 to CRC-SCs isolated from tumor stimulates differentiation and apoptosis via activation of phosphoinositide 3-kinase (PI3K) and PKB (protein kinase B)/AKT signal transductions (43). Due to the increase of oxaliplatin and 5-fluorouracil antitumor activity and promoting chemosensitization of CRC-SCs induced by BMP4 can also be accounted as an appropriate therapeutic factor against CSCs in colorectal cancer (43). Like BMP4, BMP2 plays a vital role in colon cancer remission through stimulating differentiation and sensitivity of CRC-SCs associated with possible interaction between expression levels of β-catenin and BMP2 during the progression of colon cancer (47, 48). The findings of several studies showed that some appropriate therapeutic markers in the treatment of colorectal cancer could be influenced by the BMP signaling-mediated expression levels of BMP2 (47). In line with this, it has been shown that *Abrus* agglutinin (AGG), a plant lectin, promotes differentiation of HT-29-derived colonosphere and removes tumour growth by increasing and decreasing gene expression of BMP2 and β-catenin, respectively (49). Thereby resistance of these cells is also decreased across the

effects of antitumor activity of combinations such as oxaliplatin and 5-fluorouracil (49). Furthermore, in a study, pharmaceutical combinations such as statins, as drugs of lowering cholesterol, induced downregulation of DNA methyltransferase (DNMT)-mediate demethylation of BMP2 promoter stimulate differentiation of CRC-SCs and reduce cell proliferation and stemness (50, 51). In contrast with these results, BMP2 is also known as an oncogene that has a potential function in the constitution of the colonosphere, stemness of CRC-SCs, and eventually metastasis.

Identifier factors of intestinal/colon SCs and CRC-SCs

As mentioned before, many studies have been performed to characterize the properties of intestinal SCs and CRC-SCs (52). Similarly, studies have been designed for discovering the exact number and position SCs in the different intestinal models, including +4 position and stem cell zone (53). In the +4-position model, the intestinal SCs are just above the Paneth cells and can divide asymmetrically and differentiate into the progenitor cells (54). However, the intestinal SCs in the stem cell zone named crypt base columnar (CBC) are scattered and located at the bottom of the crypt (54). On the other hand, intestinal SCs markers, including RNA-binding Musashi-1 (Msi-1) and a transcriptional repressor called Hes-1, are expressed by supposed SCs at the crypt base (55). To determine β1-integrin (CD29) as a candidate surface marker of SCs, indirect immunofluorescence showed that its expression increased in the lower one-third of colon tissue-isolated crypt cells (56). Also, staining isolated crypt cells with β1-integrin antibodies demonstrated that these cells significantly could promote clonal growth (56). Another potential intestinal marker for SCs is the B subclass of the Eph receptor that regulates Wnt-induced EphB2/Eph3 signaling in stem/progenitor cells (57). Findings of

one study suggested that any disorder in compartments of EphB2/Eph3 signaling lead to a reduction of cell proliferation and migration in stem/progenitor cells without changing the number of SCs at the crypt base (58). In another study, to consider possible polycomb complex protein Bmi-1 as an intestinal SCs marker and Wnt target gene was tracked using two integrated cassettes including internal ribosome entry site (IRES)-Cre recombinase-estrogen receptor-binding domain (ER) [*IRES-Cre-ER*] into 3' *Bmi-1* locus and β-galactosidase (*LacZ*) into the *Rosa26* locus [*Rosa26-LacZ*], isolated crypt and villi cells and tamoxifen for treatment (59). After tamoxifen injection, obtained results from the study showed that tamoxifen stimulates gene expression of *Cre* recombinase-mediated over gene expression of *Bmi-1* and *LacZ* in crypt cells (59). High LacZ activity in tamoxifen-induced labelled cells means most cells (86 cells) expressing Bmi-1 and their progeny are located above the Paneth cells. In contrast, fewer cells (5 cells) expressing Bmi-1 are located among Paneth cells, suggesting that Bmi-1 could be a potential factor for maintaining intestinal homeostasis (59). As same as Bmi-1, Lgr-5 are also considered as assumed the marker and Wnt target gene of the intestinal SCs (60). In this regard, using two knock-in allele cassettes, including *Lgr5-EGFR-IRES-CreERT2* and *Lgr5-LacZ*, an integrated cassette into *Rosa26* locus in the form of *Rosa26-LacZ*, isolated crypt and villi cells, and tamoxifen, to determine Lrg-5 as a key Wnt target gene of SCs was established (61). The study showed that tamoxifen activates the expression of CreERT2-mediated LacZ activity and eventually promotes *Lgr5*-expressing cells that were located between the Paneth cells (61).

Any disorders in genetic and epigenetic contents of colonic cells, specifically SCs, lead to colon SCs converting to a series of cells named CRC-SCs that have self-renewal,

recurrence, metastasis, therapy resistance. As regards CRC-SCs comprise a small population of tumor-derived cells. These cells play an important in different stages of colonic tumorigenesis, including initiation, growth, and maintenance of tumor. However, identification, tracking and isolating CRC-S during colon carcinogenesis is difficult due to insufficient experimental techniques and their complicated biological properties. Therefore, various presumptions based on molecular markers have been established in several experimental studies. These specific molecular factors, including CD24, CD44, CD133, CD166, CD29, CD26, CD166, Nanog, Oct-4, SOX-2, Lgr-5, ALDH-1, and EpCAM that have been initially proposed as key components of CRC-SCs. In the following sections, we discuss the prognostic value and some effects of these markers in regulating CRC-SCs self-renewal and survival in patients with CRC.

CD24 marker

CD24 marker is a small glycoprotein that is crucial for transferring extracellular messages inside of cells through binding its phosphatidylinositol domain of the cell membrane (62). CD24 is an adhesion molecule, potentially plays a key role in the metastasis of tumoral cells (63). Due to the altered clinicopathological properties and phenotype of CD24-expressing cells, CD24 could be considered a candidate marker CSCs. In the same direction, it has been reported that CD24-expressing colon cancer cell lines stimulate increasing tumor stage/grade, increasing systemic metastasis, and median patient survival (64). Changes in CD24 expression in colon cancer cell line-derived tumors also result in the secretion of extracellular and intercellular components-mediated alterations of tumor conditions. Over-expression of CD24 in human CRC tumors-derived cells

demonstrated that 92.5% of cytoplasmic expression of $CD24^+$ SW480 cells induce cell proliferation and tumor growth by activation of extracellular signal-regulated kinases Raf-1 and p38 mitogen-activated protein kinase (MAPK) signalling (65). These cells also stimulate the EMT process and eventually metastasis via over activation of TGF-β signalling-mediated overexpression of notch1 ligand (65). A study to analyze CD24 expression on a tissue microarray of 523 colorectal adenocarcinomas showed that high expression of CD24 in 50.5% of colorectal adenocarcinoma tissues promoted differentiation of colon cancers derived from colonic tumors (64). Findings of another study have demonstrated that 30% of CD24-expressing tumor cells were significantly correlated with therapeutic methods, including chemotherapy and surgery, whereas it has no significant correlation with patient survival (66). Also, $CD24^+$ cell lines such as SW480 induce a high frequency of side population (SP) phenotype, high survival in comparison with $CD24^-$ SW480 cell lines (67). Another study has identified that 84.4% of cytoplasmic CD24-expressing HT-29 cell lines compared to 68.7% of membranous CD24-expressing HT-29 promoted high tumor stage, systematic metastasis, and tumor grade (63). As regards CD24 expression in a small fraction of colorectal cancer cell lines population, including HCT-116 and SW480, this $CD24^+$ subpopulation is more capable of enhancing stemness, chemotherapy resistance, and tumorigenic capacity than $CD24^-$ subpopulation (67). It has been shown that CD24-expressing CSC population derived from cell lines could form clonogenicity in serum and SF medium. However, it has been shown that after injection of $CD24^+$-$CD44^+$ and $CD24^-$-$CD44^+$ to the NOD/SCID mice, there were no differences in tumorigenicity in both serum-containing and SF medium (67). It has been proposed that co-expression of CD24 and CD44 in CRC cell lines show CSC

characteristics (67). In contrast with these results, $CD24^+$ cell lines such as COLO205 are not capable of forming spheroid compared to $CD24^-$ COLO205 (67). These results can provide strong evidence for considering the possible CD24 marker as CSCs in different stages of CRC.

CD44 marker

CD44 CSC marker is a transmembrane glycoprotein that plays a critical role in various cellular processes, including cancer initiation, invasion, and metastasis (68, 69). In addition, CD44 is an adhesion molecule and plays a key role in tumour cell aggregation and finally, tumor growth (70). Various studies have been conducted for the identification and isolation of putative CD44-expressing cells as CSCs. In line with this, analyses of CD44 presence in primary tumors-derived cells using immunohistochemistry showed that CD44-expressing cells could promote clonal formation and tumorigenicity in xenograft tumors (71). On the other hand, inhibiting CD44+ in these cells using lentiviral RNA interference demonstrated that this could prevent and remove clonal formation and tumorigenicity (71). Furthermore, in immunohistochemistry analysis of the anatomical distribution of CD44-expressing cells, it has been shown that these cells are located at the bottom of the crypt in normal colonic tissue (72). On the other hand, many studies have been reported that the position of CD44 is a possible marker and is mainly located in the cell membrane of cells derived from the tumour.

Moreover, It has been identified that the CD44 CSC marker is highly expressed in colorectal adenomas-carcinoma, associated with clinicopathological factors such as tumor size, depth of invasion, and lymph node involvement (64, 73). Statistical analyses

between CD44-expressing cells and clinicopathological factors demonstrated a highly significant correlation between $CD44^+$ CRC cells and increased infiltration of lymphocyte, lymphovascular invasion, peritumoral budding, lymph node grade and metastasis (74). In contrast with the previous study results, another study showed that CD44-expressing CRC cells were negatively correlated with clinicopathological factors, including lymphocytic invasion, tumor, and node metastasis (75). Furthermore, it has been shown that co-expression of CD44 and cellular prion protein (PrPc), as a pathogenic factor, in CRC cells significantly promote the risk of liver metastatic via over-activation of EMT and MAPK1 signaling pathway in CRC (76). Conversely, statistical analysis for free survival rate in two different expression levels of the CD44 group showed that high expression levels of CD44 in the hepatic metastasis derived from CD44-expressing CRC cells reduce the free survival rate compared to low expression CD44 in these cells (76).

In a study, Wielenga et al. investigated the correlation between expression of CD44 and genetic defects such as *APC* or *Tcf-4* both in the intestinal mucosa of mice and humans. They showed that the CD44 marker is highly expressed in adenomas and invasive carcinoma associated with mutation of mice's *APC* (77). In addition, it has been shown that splice variant exon v6 of CD44 (CD44v6) is highly expressed in tumorigenesis of colorectal, which has a role in the prognosis in CRC (78). These results clearly show that putative CD44, as a CRC-SCs prognostic marker, plays a crucial role in identifying colonic SCs.

CD166 marker

CD166 is an activated leukocyte cell adhesion molecule (ALCAM) and possibly plays an important role in the progression of several aggressive diseases, including CRC (79). For the identification of pattern CD166 expression as an assumed marker, many studies have been conducted. Using the different experimental methods,s including specific antibodies and immunohistochemical staining, the accurate spot of CD166 marker in both normal intestinal and colon has been detected. Based on this, the different CD166 expression pattern in epithelial cells both colon and intestinal has been determined (80). Th166 expression is on the cellular surface of normal epithelial cells at the base of the crypts in both colon and intestinal (80). On the other hand, membranous and cytoplasmic high expression of CD166 in human adenocarcinoma and liver metastasis tumors were identified using antibody staining of CRC tumor tissues (81). Furthermore, a preneoplastic intestinal cancer mouse model for $Apc^{Min/+}$-induced intestinal tumorigenesis has shown that predominant cell surface and diffuse cytoplasmic of CD166 is also expressed at CD166-expressing crypt-base columnar epithelial cells that display a similar pattern of CD166 expression in comparison with human colorectal tumors (80). In further support of different localization expressions of CD166 in CRC tumors-derived cells, results of a study also demonstrated that 71.1% cytoplasmic and 34.7% membranous of CD166 is expressed in colectomy samples of patients with CRC (81).

Regarding the heterogeneous nature of CD166 expression in different cellular sections, a significant correlation between CD166-positive cells and clinicopathological factors including tumor stage and grade types, tumor location, survival, self-renewal, and vascular invasion has been examined (81). In a study, it has been reported that membranous CD166-positive cells are significantly correlated with 54.8% of the right

colon and 24.3% of left colon (tumor location) in colectomy specimens of CRC patients (81). Furthermore, it has been reported that 39.2% of loss of membranous CD166 expression widely promotes advanced T classification, lymph node metastasis, tumor border configuration, and worse overall survival than over-expression of membranous CD166 (82). In another study, interaction between sclerostin domain containing-1 (SOSTDC1) protein, an enhancer factor for tumor metastasis, and ALCAM/CD166 in metastatic CRC cells have been investigated by immunoprecipitation and mass spectrometry analyses (83). Confirmation of experimental analyses using confocal microscopy and competition ELISA showed that the N-terminal region of SOSTDC1-mediated interaction ALCAM/CD166 can stimulate invasion and liver metastasis of mouse CRC cells through activation of Src and PI3K/AKT transductions as well as inhibiting of BMP4 signaling in invasive properties of SW620, KM12SM, and KM12L4 cell lines (83). Weichert et al. investigated the membranous of ALCAM expression in CRC and the correlation between ALCAM and survival patients by immunostaining model in CRC patients. They demonstrated that both cytoplasmic and membranous AM expressions are highly expressed in 58.6% and 30.6% of cases. They also reported the relationship between the ALCAM expression and clinicopathological factors associated with shorter patient survival (84). According to Lugli's study, the prognostic effect of CD166 CSC marker in CRC by immunostaining model in primary colon cancer and normal mucosa samples and colon cancer cell lines has been examined. They demonstrated that loss of CD166 expression could increase the invasive and progressive potential of the tumor and worse survival in these patients (82). These results suggest

that CD166 as a putative prognostic marker of CRC-SCs could be a potential therapeutic target in different stages of CRC.

CD133 marker

CD133 is another stem cell surface antigen widely used as a potential marker to identify and isolate CSCs in different malignancies, including CRC (85). To investigate CD133-expressing CRC cells as CRC-SCs due to alteration of clinicopathological properties by these cells, using semiquantitative scoring of immunohistochemical staining identified that 50% or more of CD133-expressing membranous tumor cells are positively stained and considered as CD133-high (86). According to Horst's study, $CD133^{+}$ cells significantly promote tumor grade and low survival in patients with CRC (86). To evaluate CD133-expressing cells derived from 200 colorectal polyp and adenoma samples using immunohistochemical staining showed that CD133+ cells staining is positive in 17.9% of colorectal adenomas samples, which significantly enhance tumor size and differentiation degree compared to 57.1% staining of cancerous polyps (87). To track the cellular distribution of CD133 in these cells using immunohistochemical staining displayed that CD133+ is located on the luminal cell surface but not in the cytoplasm (87). Under staining CD133-expressing cells using polyclonal antibodies such anti-human CD133, CD133 expression is exclusively located on the membrane of CRC gland cells in 18 of the 20 primary CRC gland cells (88). It has been investigated that CD133+ is expressed in >3% of cells derived from samples of lymphatic invasion which can stimulate spheroid formation compared to samples containing <3% of CD133+ cells (89). An analysis of immunochemical staining in spheroids-forming two sorted subpopulations from SW620

cell lines, including CD133+ cells and CD133- cells, showed that CD133 is positively expressed in two spheroid groups that were associated with the spheroid formation in them (88).

Due to the high resistance of $CD133^{+}$ cells against chemotherapeutic drugs, these cells may also be influenced tumorigenesis process. Regarding the role of CD133-expressing cells in tumorigenesis and tumor growth, analyses of flow cytometry demonstrated that tumorigenesis and chemoresistance against 5-FU are significantly higher in $CD133^{+}$ cells compared to $CD133^{-}$ cells (89). Furthermore, several studies showed that $CD133^{+}$ colon cancer cells stimulate tumor initiation in a small subset of colon cancer cells. According to Shmelkov's study, $CD133^{+}$ cells are expressed in colon epithelial cells and metastatic transition (90). Moreover, it has been shown that the expression of CD133-expressing cells was positively correlated with CRC with hepatic metastasis and clinicopathological factors, including patient survival (91, 92). Furthermore, Lin et al. reported that the over-expression of $CD133^{+}$ cells in the peripheral blood of patients with CRC was associated with the progression and recurrence of CRC (93). In contrast, it has been demonstrated that the low expression of CD133 is significantly correlated with CA 19-9 serum levels and advanced tumor stage in primary CRC tissue (94). These results and findings suggest that the CD133 marker could be a prognostic target for therapeutic interventions in patients with CRC.

Lgr5

One of the Wnt pathway targets is a seven-transmembrane protein known as Lgr5, which can recognize intestinal stem cells (95). Lgr5-regulating cells can differentiate into various

cell types in the intestinal tract as intestinal stem cells, including goblet, Paneth and entero-endocrine cells (95). For instance, functional LGR5+ cells can produce a transient population of inactive Paneth cell progenitors regulating both LGR5 and four markers, which, in case of damage, obtain the features of an active stem cell population (96). Additionally, Lgr5 increases the canonical Wnt pathway via providing receptors for R-spondin1-4, which, through coupling to Lgr5, elevates LRP6 phosphorylation(97). Kemper and colleagues demonstrated that up-regulation of Lgr5 stimulates clonogenic growth and has been suggested as a selective biomarker for human CRC-SCs (98). It has been determined that germline variations in the CSC-related genes LGR5 are associated with shortened recurrence time in CRC patients at stage II/III (99).

Therapeutic approaches against markers-expressing CRC-SCs

Due to some standard phenotypic and functional features between CRC-SCs and normal colon SCs, it is difficult to identify and isolate them effectively. Therefore, from identifying and isolating marker-expressing cells to proposing them as CRC-SCs, it is necessary to find appropriate therapeutic strategies, including monoclonal-polyclonal antibodies, pharmaceutical elements, chemotherapy and radiotherapy drugs, and natural compounds. In the current review, we summarize some of these novel therapeutic approaches that could influence the identification of CRC-SCs and help reduce the incidence of CRC and management of patients with CRC.

A traditional Chinese medicinal compound named atractylenolide 1 (ATL-1), as an active ingredient, has been investigated its inhibitory effects on CRC. Regarding the inhibitory effects of ATL-1, the findings of a study showed that ATL-1 also inhibit metastasis of CRC-

SCs by reducing miR-200c activity in these cells. On the one hand, the mechanism of the inhibitory effect of ATL-1 on miR-200c expression and metastasis of CSCs-isolated extracellular vesicles (EVs) has been examined. It was shown that ALT-1 could suppress metastatic activity by reducing proliferation and stimulating apoptosis caused by the low expression of miR-200c in EVs derived from CSCs (100). On the other hand, it has been demonstrated that ALT-1 can also be influenced on reducing invasion and stemness of CSCs-derived EVs through suppressing PI3K/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling cascades (100).

Caffeic acid is a primary bioactive coffee component, and its potential inhibitory effects and molecular mechanisms on multiple cancers such as CRC have also been determined. Consistent with findings of a study, expression levels of CD44+ and CD133+ subpopulation in caffeic acid-treated CSCs derived from HCT-116 cell lines is lower than non-treated cells (101). According to the inhibitory effects of caffeic acid and using a transwell invasion assay has been demonstrated that caffeic acid can reduce cell migration and tissue regeneration via decreasing expression levels of matrix metalloproteins 2/9 (MMP-2/9) in HCT-116-derived CSCs treated by caffeic acid (101). To evaluate the role of caffeic acid inhibitory function in self-renewal and sphere formation using two specific Akt activator SC79 and Akt inhibitor V showed that Akt inhibitor synergically inhibits sphere formation and self-renewal of CSCs compared to stimulatory effects of PI3K/Akt signaling induced by Akt activator in caffeic acid-treated cells (101).

Celecoxib belongs to the nonsteroidal anti-inflammatory drugs (NSAIDs) family and has the most efficacy on antitumor growth through underlying mechanisms dependent on cyclooxygenase 2 (COX-2) inhibition (102). The findings of several studies have shown

that the inhibitory effect of celecoxib can widely be influenced on the CRC-SCs population (103). Similarly, it has been demonstrated that celecoxib can also attenuate tumor growth and CSC property of CRC cells by inhibiting c-Met expression in these cells (104). Considering the role of celecoxib-inhibited c-Met in CRC cells, it has been observed that celecoxib inhibits the spheroid formation and CRC-SCs property through the low expression of c-Met in some of the CRC cell lines, including COLO320DM, HT-29, and HCT-116 (104). Across from an inhibitory function of celecoxib, a pro-inflammatory mediator named PTGS2-derived prostaglandins E_2 (PGE_2) can widely stimulate most of the cellular processes of CSCs, including tumor cell proliferation survival, migration, invasion, and metastasis through activation of several signaling transductions (105). Furthermore, due to the positive correlation between mRNA levels of PGE2 and CSC-associated markers, including CD133, CD44, LGR5, and SOX2, it has been shown that PGE_2 may be able to promote cell proliferation of these cells in human colon carcinoma at grade II-III (105). In this regard, identification of PGE_2 effect on spheroid formation using IHC and flow cytometry techniques indicated that PGE_2 could promote tumor burden, tumor SCs and spheroid formation with expression levels of CD133 and CD44 in colonic tumor cells derived from PGE2-treated *APC* $^{Min/+}$ mice (105).

Conclusion

This review summarizes the recent findings on the clinical implications of CSCs in the pathogenesis of CRC. Results support the hypothesis that makers-expressing CRC cells and target genes mediated by aberrant activation of the several signal transductions in these cells could be possibly considered as CRC-SCs-like cells. Therefore, these markers

and target genes could be putative diagnostic factors and clinically valuable markers for the different stages of CRC.

Due to the positive correlation between CSCs and pathological conditions in CRC, it is suggested that the expression of markers in these cells could be potential targets for therapeutic interventions in this disorder. In the context of these experimental results regarding the location and number of CRC-SCs and the expression level of markers, further studies are needed to determine these markers' accurate underlying signalling mechanisms. These can act as suitable therapeutic targets for the greater understanding of the identification and isolation of CSCs and, eventually, the more effective management of patients with CRC.

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