Self-renewal molecular mechanisms of colorectal cancer stem cells

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Abstract. Colorectal cancer stem cells (CCSCs) represent a small fraction of the colorectal cancer cell population that possess self-renewal and multi-lineage differentiation potential and drive tumorigenicity. Self-renewal is essential for the malignant biological behaviors of colorectal cancer stem cells. While the self-renewal molecular mechanisms of colorectal cancer stem cells are not yet fully understood, the aberrant activation of signaling pathways, such as Wnt, Notch, transforming growth factor-β (TGF-β)/bone morphogenetic protein (BMP) and Hedgehog-Gli (HH-GLI), specific roles mediated by cell surface markers and micro-environmental factors are involved in the regulation of self-renewal. The elucidation of the molecular mechanisms behind self-renewal may lead to the development of novel targeted interventions for the treatment of colorectal cancer.

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1. Introduction

Colorectal cancer (CRC) is the second most common type of cancer and the fourth leading cause of cancer-related mortality worldwide, occupying approximately 9.7% of the total cancer cases and 8.5% of the number of deaths in 2012 (1). CRC is composed of heterogeneous cell populations differing in gene expression and tumorigenicity (2,3). Sporadic CRC and hereditary CRC both originate from the stem cell stage. CRC stem cells (CCSCs) represent a small fraction of the CRC cell population with self-renewal and multi-lineage differentiation potential and the ability to drive tumorigenicity (4).

It is thought that CCSCs originate in three different ways: first, they may be derived from the malignant transformation of normal colorectal stem cells. Colorectal stem cells have the ability to proliferate and self-repair. Gene mutations can further accumulate during their long survival. Evidence has demonstrated that colorectal stem cells become tumorigenic more easily (4-6). Barker et al suggested that only Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5)+ stem cells, in cooperation with APC-deficiency, may lead to colorectal adenoma formation (7). The stem-like Lgr5+ tumor initiating cells located in the base of adenomas are similar to normal stem cells (8). In the initiation process of CRC, normal colorectal stem cells acquire oncogenic mutations through the interaction between internal and external factors. Subsequently, in the evolution of CRC, the heterozygous loss of APC, DCC and p53 occurs, accompanied by DNA damage, DNA-repair mutations and altered methylation status (9,10). Second, CCSCs may originate from the dedifferentiation of common cancer cells. Cells with certain differentiation characteristics, such as progenitor cells or mature cells, acquire stemness by dedifferentiation. The successful induction of induced pluripotent stem cells (IPS) has demonstrated that differentiated cells, even in the stage of terminal differentiation, can regain stemness through a reset by certain specific regulation factors. Transducing transcription factor Oct3/4, Sox2, c-Myc and Klf4 into mouse fibroblast cells can drive cells to dedifferentiate and acquire stemness (6). Schwitalla et al indicated that increasing nuclear factor-κB (NF-κB) signaling in intestinal epithelial cells would activate the Wnt signaling pathway, thus eliciting dedifferentiation and promoting tumorigenicity (11). Third, CCSCs may originate from cell malignant transformation through the influence of the micro-environment. The transformation of non-cancer stem cells to cancer stem cells is dependent on transforming growth factor-β (TGF-β) signaling in the micro-environment, and the process is most likely relevant to epithelial-mesenchymal transition (EMT) (12,13). Mani et al found that mammary gland cells undergoing EMT by Snail or Twist induction regained stem cell markers and the ability to self-renew (14).

CCSCs are heterogeneous, as they contain various subpopulations or are in different stages of stem cell development (2). B-cell-specific Moloney murine leukemia virus insertion
site 1 (Bmi1)+ quiescent cancer stem cells are insensitive to high-doses of radiation, while Lgr5+ active cancer stem cells have a strong homeostatic regeneration ability (15). If the latter become injured or destroyed, the former can mobilize to transform into an active status. Hence, quiescent cancer stem cells most likely function as a reservoir to maintain the homeostasis of stem cells. The micro-environment dictates the balance between them (15,16). At present, therapy for CRC targets mainly active cells, while quiescent stem cells can escape, leading to relapse and resistance to treatment.

CCSCs are similar to normal adult stem cells as regards biomarkers (Table I). Consequently, three methods have been developed to isolate CCSCs: the first is dependent on cell surface markers. CCSCs can be isolated by FACS based on CD133+ (17,18), CD44+CD24− (19), CD44+CD58+ (20) and CD166+ (21,22). The second is dependent on the characteristic of specific enzymes, such as aldehyde dehydrogenase 1 (ALDH1) (23) and ATP-binding cassette subfamily G member 2 (ABCG2) (24). The third is culturing the cells in serum-free, low-adhesion conditions (25). The methods for identifying CCSC properties include evaluating the ability of continuous sphere formation in vitro, continuous tumorigenicity in NOD/SCID mice and the similarity of differentiation between xenograft tumors and primary tumors.

The characteristics of CCSCs are regulated by different mechanisms. Self-renewal is a fundamental feature of the malignant biological behaviors of CCSCs. Several pathways, cell surface markers and micro-environmental factors are involved in CCSC self-renewal.

2. Pathways involved in the self-renewal of CCSCs

The aberrant activation of signaling pathways plays important roles in the evolution and progression of CRC. The Wnt, Notch, bone morphogenetic protein (BMP)/TGF-β, Hedgehog-Gli (HH-GLI), epidermal growth factor receptor (EGFR)/mitogen-activated protein kinase (MAPK), NF-κB and Akt/mTOR pathways are involved in the self-renewal of CCSCs (Fig. 1).

Wnt pathway. The Wnt pathway is one of the most important pathways in the tumorigenesis and progression of CRC. Over 90% of CRC cases display an over-activation of Wnt signaling (26). According to whether this activation is dependent on transcriptional regulation by transporting β-catenin into the nucleus, the Wnt pathway is divided into the canonical pathway (β-catenin-dependent) and the non-canonical pathway (β-catenin-independent). The canonical pathway mainly consists of extracellular signaling proteins (Wnt1, Wnt3a, Wnt8, etc.), the transmembrane receptor Frizzled (Fzd), co-receptor low-density lipoprotein-related receptor 5/6 (LRP5/6), Dishevelled (Dsh), β-catenin, axis inhibitor (Axin) and the intranuclear transcription factor T-cell factor (TCF)/lymphoid enhancer factor (LEF). In the absence of Wnt, β-catenin interacts with Axin, adenomatous polyposis coli (APC) and glycogen synthase kinase-3β (GSK-3β) to form a destruction complex and is phosphorylated by GSK-3β. Phosphorylated β-catenin recruits ubiquitin E3 β-transducin repeat containing protein (β-TrCP) and is then degraded by the proteasome, thus maintaining cytoplasmic β-catenin at a relatively low level (27-29). With Wnt signaling, the Wnt protein binds to the Fzd-LRP complex and recruits Dsh to the cytomembrane. The induction of phosphorylation by Dsh separates GSK-3β from Axin and inhibits the formation of the Axin-GSK-3β-APC complex, inhibiting the phosphorylation and ubiquitination of β-catenin. Free β-catenin accumulates in the cytoplasm and translocates to the nucleus, targeting LEF and TCF (30). These proteins promote the transcription and expression of downstream targets, such as c-Myc, cyclin D1 and Axin2 (31). Disrupting the β-catenin/TCF-4 activity of CRC cells induces a rapid G1 arrest and blocks the proliferative compartment in colon crypts from genetic programming.

The suppression by c-Myc on the promoter of the cell cycle inhibitor p21 plays an important role in this process. Evidence from conditional gene deletion of c-Myc suggests that c-Myc(-/-) crypt cells are smaller in size and have a slower cycle compared to wild-type cells and c-Myc-deficient crypts are prone to loss and can be replaced by c-Myc-proficient crypts within weeks (32,33).

In recent years, many other non-classical Wnt proteins have been discovered, establishing a much more complex and precise regulatory network for the canonical Wnt pathway. YAP/TAZ appears to be an important component of the β-catenin destruction complex. In the absence of Wnt, YAP/TAZ recruits β-TrCP to the complex and degrades β-catenin, blocking pathway activity. When the Wnt pathway is activated, YAP/TAZ is released from the complex so that β-catenin can accumulate in the nucleus to stimulate downstream effectors (34). Polycomb group protein Bmi-1 activates Wnt signaling by upregulating the transcription of Wnt factors Wnt3A, Wnt7A, Wnt7A, Wnt10A and Wnt4 by or by repressing the DKK family. The Wnt downstream target c-Myc in turn promotes the transcription and trans-activation of Bmi-1, forming a positive feedback loop (35). Oncogenic transcription factor MYB cooperates with β-catenin to co-stimulate c-Myc expression (36).

High Wnt activity can define the CCSC population functionally. CRC cells with high Wnt activity upregulate the expression of the stem cell-associated genes, Lgr5 and achaete-scute family bHLH transcription factor 2 (ASCL2), while ones with low Wnt activity upregulate the expression of the epithelial differentiation-associated genes, mucin 2 (MUC2), keratin 20 (KRT20) and fatty acid binding protein 2 (FABP2) (37). CD133+, CD24+CD29+ or CD44+CD166+ cells exhibit high Wnt activity (37). High Wnt activity is associated with high clonogenic cancer stem cell potential, while low Wnt activity is not (37). Similar evidence defines the association between Wnt activity and CRC cell proliferation and EMT (38).

Non-canonical Wnt signaling mainly consists of the Wnt/Ca2+ pathway and JNK-mediated planar cell polarity (PCP) pathway. Under different conditions, non-canonical Wnt pathways function synergistically or antagonistically with the canonical Wnt pathway. The Wnt/Ca2+ pathway is activated by the interaction between the Wnt-Fzds complex and co-receptor Ror1/2. They activate phospholipase-C (PLC) to generate 1,2-diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3), facilitating the accumulation of Ca2+ in the cytoplasm. The increase in Ca2+ level activates calcinulin (Cn), calmodulin-dependent protein kinase II (CaMKII) and protein
Table I. The comparison of biomarkers between normal stem cells and CCSCs.

| Marker (Ref.) | Property/function |
|---------------|-------------------|
| Normal colorectal stem cells | CD29 (140) Cell adhesion molecule |
| | DCAMKL-1 (141) Kinase |
| | Lgr5 (106) Component of the Wnt receptor complex |
| | Msi-1 (142) RNA-binding protein |
| | ALDH1 (23) Detoxifying enzyme |
| Colorectal cancer stem cells | Bmi1 (143) A polycomb group protein that regulates gene silencing |
| | CD24 (144) Cell adhesion molecule |
| | CD29 (87) Cell adhesion molecule |
| | CD44 (91) Cell adhesion molecule |
| | CD49f (145) Cell adhesion molecule |
| | CD58 (20) Cell adhesion molecule |
| | CD66c (146) Cell adhesion molecule |
| | CD133 (18) Glycoprotein, the most classic marker of CCSCs |
| | CD166 (21,22) Cell adhesion molecule |
| | DCLK1 (147) Kinase |
| | EpCAM (22) Cell adhesion molecule |
| | Lgr5 (106) Component of the Wnt receptor complex |
| | Msi-1 (142) RNA-binding protein |
| | OLFM4 (148) Glycoprotein |

CCSCs, colorectal cancer stem cells; Lgr5, Leucine-rich repeat-containing G protein-coupled receptor 5; Msi-1, Musashi-1; ALDH1, aldehyde dehydrogenase 1; DCAMKL-1, doublecortin and CaM kinase-like-1; DCLK1, doublecortin-like kinase 1; EpCAM, epithelial cell adhesion molecule; OLFM4, olfactomedin-4.

Figure 1. Pathways involved in the self-renewal of colorectal cancer stem cells (CCSCs).
kinase C (PKC), which further activate the transcription factor nuclear factor of activated T-cells (NFAT), NF-κB and cAMP response element binding protein (CREB) (39). In addition, Wnt5/Ror signaling can also generate ubiquitin ligase Siad2, calpain and caudal type homeobox 2 (CDX2). CDX2 acts as a transcription factor to regulate the downstream targets (39). Wnt5α acts differently as a proto-oncogene or tumor suppressor in different tissues. With respect to CRC, MacLeod et al found that Wnt5α can generate Siad2 and promote β-catenin phosphorylation and degradation, which inhibit the growth of cancer stem cells (40). PKC can phosphorylate β-catenin independent of GSK-3β to facilitate degradation (41). Moreover, PKC can suppress APC phosphorylation, suggesting that PKC can inhibit colorectal cells from proliferating through the negative regulation of the canonical Wnt pathway by APC (42). The PKCα-dependent phosphorylation of retinoic acid-related orphan nuclear receptor α (RORα) on serine residue 35 can suppress the expression of target proteins of the canonical Wnt/β-catenin pathway (43). CaMKII acts upstream to activate the TAK1-NLK pathway and inhibit the DNA-binding activity of the β-catenin-TCF-4 complex through serine/threonine phosphorylation of TCF-4 (44). The Wnt/PCP pathway is mediated by Wnt (Wnt5α, Wnt11)-Fzd and Dsh. Wnt/PCP plays an important role in regulating tissue polarity and cell motility through the activation of small GTP-binding proteins, including Rac and RhoA, and protein kinases, including c-Jun N-terminal kinase (JNK), Rho-associated kinases and nemo-like kinase (NLK) (45). Van-Gogh-like 2 is an important component of Wnt/PCP, essential in establishing epithelial cell polarity. Van-Gogh-like 2 inhibits CRC through antagonizing the canonical Wnt pathway (46). By contrast, JNK/c-Jun regulates the expression of TCF4 to promote canonical Wnt signaling (47).

We recently found that nuclear receptor-interacting protein 2 (Nrip2) is a novel interactor of the non-canonical Wnt pathway. Nrip2 inhibits the transcription of HMG-box transcription factor 1 (HBP1) through the arrest of retinoic acid-related orphan nuclear receptor β (RORβ) in the cytoplasm and its subsequent degradation to promote the transcription of the downstream gene, TCF/LEF, a process connected to activating the canonical Wnt signaling pathway (unpublished data).

**Notch pathway.** Notch can also be divided into canonical and non-canonical pathways. Typical Notch ligands include Delta-like (DLL)1, DLL3, DLL4, jagged (JAG)1 and JAG2 with a Delta-Serrate-Lag 2 (DSL) domain, while atypical ligands include DNER, F3/Contactin and NB-3 without a DSL domain. When the ligand interacts with the Notch1, Notch2, Notch3 or Notch4 receptor, continuous proteolysis is triggered by γ-secretase, releasing the active Notch intracellular domain (NICD). In the canonical Notch pathway, NICD translocates to the nucleus and binds to the transcription factor, CSL. Then CSL-NICD complex is activated by Mastermind family co-activators for the transcriptional activation of targets HES1 and HEY1 to suppress differentiation and maintain stemness. Otherwise, NICD binds to nuclear p50 or c-Rel to activate NF-κB activity 9 (non-canonical pathway). Another non-canonical Notch pathway is triggered by an atypical Notch ligand to form the CSL-NICD-Deltex complex, activating MAG transcription and promoting differentiation (48). Which pathway is activated depends on the interaction between ligands and receptors. NUMB regulates intracellular Notch activity in the process of cell division, inhibiting Notch transmission in the cytoplasm (49,50). Musashi-1 (Msi-1), a conservative RNA-binding-protein (RBP), can upregulate Notch activity by inactivating NUMB (51).

In general, an aberrantly activated Notch pathway is oncogenic although anti-oncogenic partly in dermatoma and the cervical uterus (48). Notch activity in the CRC initiating cells is 10- to 30-fold higher than in colon cancer cells. Notch inhibits apoptosis of CSCCs by repressing the cell cycle inhibitor p27. In addition, Notch can maintain self-renewal and inhibit differentiation through repressing secretory cell lineage differentiation targets MUC2 and atonal homolog 1 (ATOH1) (52).

Notch1 and HES1 are involved in the malignant transformation of the normal colonic mucosa (53). HES1 increases stemness-related genes in CRC cells and leads to the over-expression of CSCC markers, such as CD133, ABCG2, Nanog and ALDH1. Additionally, HES1 increases the size of CD133 and stem-like side population cells to enhance self-renewal properties (54). The inhibition of Notch signaling by a γ-secretase inhibitor can inhibit the growth of CRC cells, suggesting a potential therapeutic target for CRC (55).

**BMP/TGF-β pathway.** The TGF-β family has over 40 members, including the TGF-β and BMP subfamily (56). When the signaling is activated, activated receptors further phosphorylate the intracellular receptor-regulated SMAD (R-Smad). Phosphorylated R-Smad interacts with Co-Smad to create a complex that translocates into the nucleus and plays a role as a transcription factor (57).

The downstream targets of TGF-β are pivotal cell cycle regulation proteins, including p21, p27 and p15. In most situations, their activation leads to growth arrest (58). Mutated inactivation occurs in at least one component of almost every CRC case, from frameshift mutations caused by microsatellite instability to mutations of Smad4 and Smad2 (59-62). Zubeldia et al injected colon adenocarcinoma cells pre-treated with TGF-β into the spleens of mice and found that it promoted primary tumor development and liver metastasis (63). TGF-β can promote EMT through inducing EMT-related transcription factors Snail1/2, Twist and zinc finger E-box binding homeobox (ZEB)1/2 and elicit cell dedifferentiation (64). Snail induces interleukin (IL)-8 expression through binding to its promoter and enhances intercellular adhesion molecule (ICAM)-1 expression, which further activates the TGF-β signaling pathway (65).

As regards the BMP signaling pathway, mutations in the BMP receptor BMPRIA and Smad4 lead to juvenile intestinal polyposis and Cowden disease, respectively (56). The down-regulation of BMP3 occurs in 89% of primary CRC cases. The early silencing and frequent silencing of BMP3 is crucial in CRC progression (67). BMP4 is not expressed in CRC, while it exists in differentiated cells. Recombinant BMP4 promotes the differentiation and apoptosis of CSCCs (68). Inhibiting the BMP pathway with Dorsomorphin causes mesenchymal stem cells to acquire epithelial-like traits, including the expression of cytokeratin-18 and E-cadherin. The progress occurs...
through the downregulation of Snail, Slug and COX2 to affect cell motility, invasiveness and tumor growth in vitro (69). Moreover, in CRC initiation, BMP signaling maintains the balanced control of stem cell self-renewal by inhibiting the Wnt pathway. The zinc-finger transcription factor, GATA binding protein 6 (GATA6) is a crucial regulation factor connecting the Wnt and BMP pathways. Competing with β-catenin/TCF4, GATA6 binds to a distal regulatory region of BMP4, decreases the threshold of the BMP pathway for CCSC expansion and inhibits stem cell differentiation (70).

**HH-GLI pathway.** It has been demonstrated that the self-renewal ability of CCSCs is dependent on HH-GLI activity in vivo (71). HH proteins initiate signaling through binding to transmembrane receptor PATCHED1 (PTCH1) and relieve its inhibition to GPCR-like protein Smoothened (SMO). SMO then localizes to primary cilia and the signaling transduces through several parts to finally mediate the three GLI zinc finger transcription factors, enhancing the GLI1 function and inhibiting GLI repressors. GLI code (the sum of all functions of the three GLI proteins) transforms into an activating state and triggers the expression of target genes such as PTCH1, GLI1, HIP, many of which are components of the HH-GLI pathway. Varnat et al found that CRC cells exhibited a high activity of HH-GLI signaling and located active HH-GLI signaling in SHH+/PTCH1+/GLI1+ tumor cells rather than the stroma. They inhibited HH-GLI signaling by RNAi or cyclopamine treatment and tested the influence on CRC cell growth, recurrence and metastases. The results suggested that the growth, recurrence and metastases of CRC xenografts required HH-GLI activity and cells with high HH-GLI activity owned stronger EMT potentials. Furthermore, they injected CD133+ cells infected with lentivirus encoding shSMO or shPTCH1 into nude mice, isolated tumor cells after 2-3 weeks and subsequently analyzed the ratio of CD133+ subpopulation to test stem cell behaviors in vivo. The results suggested that the self-renewal of CCSCs relied on the direct function of HH-GLI activity in vivo (71).

Components of the HH-GLI pathway may also influence CRC progression by connecting to the Wnt pathway. The role is controversial. GLI1 can downregulate the level of β-catenin, the expression of c-Myc and proliferation ability (72). However, SMO significantly increases in the intestinal adenoma of Apc(+/-) mice and knockdown of SMO in human CRC lines inhibits cell proliferation. In Apc(+/-)Delta716)SMO(+/+/-) mice, the number of large polyps decreases, poly morphology recesses and proliferation of cancer cells reduces. It is further demonstrated the decreased expression of SMO attenuates the β-catenin-dependent transcription instead of HH-responsive GLI-dependent transcription and SMO promotes tumorigenesis through Wnt signaling (73).

**NF-κB pathway.** The NF-κB pathway regulates immune-inflammatory responses, cell survival and proliferation, playing an important role in tumor formation. Members of the NF-κB family often display as dimers, in a quiescent condition, NF-κB binds to IκB to form a heteromultimer, residing in the cytoplasm in an inactive state. With the stimulation of NF-κB activators, IκB kinase (IKKs) are activated, leading to triopolymer phosphorylation, ubiquitylation of the N-terminus of the IκB protein and degradation. Thus, the NF-κB dipolimer is released and is further activated through post-translational modifications. The NF-κB dipolimer then translocates to the nucleus to interact with κB sequences and regulates transcription (74).

The CCSC self-renewal mechanisms related to NF-κB lie mainly in three areas. The first is related to dedifferentiation. Schwiatala et al demonstrated that increasing NF-κB recruits CREB-binding protein (CBP) and helps CBP to bind RelA/p65 to β-catenin, thus activating Wnt and inducing the dedifferentiation of non-cancer stem cells (11). The second is the induction of stem cell gene expression. IκB kinase 2 (IKK2ca) is constitutively activated in intestinal epithelial cells and increases stem-like genes ASCL2, olfactomedin 4 (OLF4M4), Delta-like 1 homolog (DLK1) and Bmi-1 in intestinal stem cells to drive intestinal tumorigenesis (75). The third is that Ikk2ca-expressing intestinal epithelial cells can secrete cytokines and chemokines, induce bone marrow cells, and activate fibroblasts, thus creating a tumor micro-environment (75).

**EGFR/MAPK pathway.** Epidermal growth factor (EGF) signals are essential for the emergence and maintenance of CCSCs (76). The interaction between EGF and EGFR promotes the dimerization and phosphorylation of receptors, thus activating a downstream signaling cascade involving MAPK, AKT and JAK-STAT (77). The MAPK pathway passes through a three-step phosphorylation to further activate downstream targets (78). EGFR promotes the expression of high levels of Musashi-1, Lgr5 and low levels of CK20 by CCSCs, facilitating rapid tumor growth. The activation of EGFR signaling promotes the expression of stem cell-related molecules Notch, Shh, Oct3/4 and Wnt. EGFR inhibitors inhibit CCSC proliferation and induce apoptosis through suppressing EGFR phosphorylation and downstream signaling proteins, such as Akt kinase and ERK kinase (76).

**Akt pathway.** The Akt cascade regulates cell survival and proliferation in many different tumors (79). Recently, a novel kinase-independent Akt pathway was discovered (80). When the Akt kinase domain is inactivated, Akt can resist a hostile environment through another domain of the Akt protein, which is named PH domain. The process may be related to the regulation of interacting protein partners. The function of PH domain may be influenced by kinase domain to get to a particular sub-cellular compartment and/or interact with specific effector proteins (80). Akt can be a bridge connecting the BMP pathway with Wnt pathway. BMP positively regulates PTEN activity to inhibit Akt pathway. The active serine/threonine kinase in the Akt pathway can activate 14-3-3-zeta in the β-catenin complex. 14-3-3-zeta contributes to the stabilization and nuclear translocation of β-catenin, thus facilitating CCSC self-renewal by activating Wnt (81).

3. **Molecular surface marker-mediated self-renewal of CCSCs**

CCSC biomarkers are putative to be related to the CCSC phenotype (Table I). Characteristic cellular surface markers, important components of CCSC biomarkers, are what we focus on in this review. Specific mechanisms of these surface markers to regulate CCSC self-renewal are shown in Fig. 2.
CD24. CD24 is a heavily glycosylated protein located on the external membrane surface, promoting the renewal of the cell cycle through increasing proliferation and suppressing apoptosis (82). CD24+ cells exhibit CCSC properties, including high chemotherapy resistance, self-renewal ability and tumorigenic potential (83). The promoter of CD24 contains a TCF/LEF sequence, suggesting that CD24 is a target of the Wnt pathway (84). In addition, CD24 activates the Src pathway and induces the expression of c-Jun and c-Fos to promote the expression of miR-21, which inhibits Pdcd4 and PTEN to facilitate CRC progression (85). Evidence from gastric cancer suggests that CD24 can maintain cell survival and promote invasion through activating STAT (86).

CD29. CD29, also known as β1-integrin, heterodimerizes with one of 12 possible α subunits (87). β1-integrin is the major integrin protein in normal cells and tumor-associated cells and is thought to play a part in tumor invasion and metastasis. Alteration of expression in integrin β1-subunit significantly correlates with lymph node metastasis and depth of invasion in CRC (88). Song et al proposed β1-integrin may induce proliferation and migration through HH-GLI pathway (89).

The involvement of β1-integrin in tumor invasion depends on the process of hypersialylation. Seales et al discovered oncogenic ras expressed in 50% of colorectal adenocarcinomas and it upregulated ST6Gal-I, a Golgi glycosyltransferase. The enforced expression of ST6Gal-I in SW48 cells has been shown to induced the sialylation of β1-integrin to upregulate attachment to collagen I and laminin and increase the association with talin, a well-accepted marker for integrin activation, which suggests that β1-integrin alters cell preference for certain extracellular matrix milieus and stimulates cell migration to promote CRC progression (87).

CD44. CD44 is type I transmembrane glycoprotein, comprised of variable spliceosomes, generating different isoforms through inserting one or more exons into a common framework region. These variable isoforms may be a critical process in CRC metastasis (90-92). CD44 expression is restricted to the crypt of the epithelia in the normal intestinal mucosa of APC-mutated mice while it is highly expressed in adenomas and invasive carcinomas (93). Although the specificity of CD44 to CCSCs is under debate, CD44 is useful in isolating CCSCs combined with other surface markers (20,21,94). The mechanism of the participation of CD44 in maintaining stemness has mainly three aspects. First, CD44 is a Wnt target gene and is involved in the Wnt-related tumorigenesis. At the level of Wnt receptor LRP6, CD44 phosphorylates LRP6 on Wnt stimulation and assists LRP6 in correct membrane localization (95). Second, internalized CD44 interacts with STAT3 and acetyltransferase p300 to form a complex, eliciting STAT3 acetylation and dimerization. The acetylated STAT3 dimer associated with CD44 translocates to the nucleus dependent on a nuclear localization signal (NLS) and binds to the promoter of cyclin D1, increasing cyclin D1 expression and cell proliferation (96). Further research demonstrates that the C terminus of the CD44 molecule is related to increased anoikis resistance in sphere-forming cultures while the N terminus contributes to the interaction with STAT3 and p300. Nuclear CD44/STAT3 signaling promotes the reprogramming of cancer cells, with increasing expression of SOX2 and OCT4. Additionally, CD44 is related to EMT (97). Third, Interaction between CD44 and pyruvate kinase M2 (PKM2) facilitates synthesizing glutathione through the pentose phosphate pathway to resist reactive oxygen species (ROS) accumulation, thus promoting tumor cell aerobic glycolysis to meet the requirements for tumor synthesis demands (98).

CD58. CD58, also known as lymphocyte function-associated antigen-3, is a heavily glycosylated protein on the cell surface and a cell adhesion molecule belonging to the Ig superfamily. CD58 is expressed on the surface of most cells in the hematopoietic system and non-hematopoietic system, including endothelial cells and epithelial cells (20). Colon cancer HT-29
and Caco-2 cells express CD58 at low levels (99). CD58 is a ligand for CD2 and their interaction functions as a specific immunity co-stimulation to promote T cells to secrete CXCL-8, which can promote the self-renewal of CCSCs (100). Our research demonstrated that CD58 promotes the maintenance of self-renewal through activating the Wnt/β-catenin pathway (20). The DKK family is comprised of inhibitors of the canonical Wnt signaling pathway. CD58 interacts with downstream DKK3 and elicits DKK3 degradation to activate the Wnt pathway (20).

**ALCAM/CD166.** ALCAM/CD166, belonging to the Ig superfamily, is highly expressed in endogenous intestinal stem cells and is related to intercellular adhesion (101,102). CD166 is a target of the Akt pathway regulated by TGF-β and NF-κB p50/p65 (103-105). Whether CD166 is a positive or negative regulator of tumor development is still under debate. Traditional opinions suggest that CD166 is associated with tumor aggression and progression, anti-apoptosis and resistance to autophagy (101,104). CD166 mediates its functions via homophilic (CD166-CD166) or heterophilic (CD166-CD6/9) interactions (102). A recent study indicated that through the PI3K/AKT pathway, CD166 regulates the downstream YAP protein to resist apoptosis, which can be promoted by the interaction between CD9 and CD166. Additionally, the interaction between PI3K/AKT signaling and CD166 can form a positive feedback loop to further promote CD166 expression (105). PI3K/AKT/CD166 regulate the expression and activity of YAP in at least two types of mechanisms. The first utilizes the downstream target CREB to regulate YAP transcription. The second mechanism is the post-transcriptional control of YAP protein stability by suppressing AMOT130 (105).

**Lgr5.** Lgr5, a G-protein-coupled receptor with a leucine rich repeat, is overexpressed in CRC cells and alters along with CRC progression (32,106). Lgr5+ cells are located in the crypt base and have the potential to generate all of the intestinal epithelial cell lineages, maintaining self-renewal and homeostasis of the intestinal mucosa (107,108). The mechanism of self-renewal regulation by Lgr5+ is through the Wnt/β-catenin pathway. With activated Wnt signaling, Lgr5+ forms a complex with Frizzled/LRP. The complex can bind to Rspomd1 (RSPO), a Wnt collaborative enhancer, to further enhance Wnt signaling (109). Recently, it has been indicated that after Lgr5 recruits RSPRO, RSPRO binds to Rnf43/Znrf3, an E3 ligase that ubiquitinates Frizzled to protect Frizzled from degradation and maintain Wnt signaling (110).

**ALDH1.** ALDH1 is reported to be a potential marker for normal or malignant CCSCs. Immunostaining has demonstrated that normal crypt bases only express a small amount of ALDH1. However, in the transformation from normal epithelia to mutant epithelia, and finally to adenomas, ALDH1+ cells increase in number and distribute more widely along the crypt axis (23). Patients with inflammatory bowel diseases with atypical hyperplasia express higher levels of ALDH1, while patients with simple inflammation exhibit no increase. This finding suggests that ALDH1 may be a critical point distinguishing atypical hyperplasia from inflammatory hyperplasia and a potential marker for pre-cancerous lesions (111).

Evidence from prostate cancer suggests that ALDH1A1 activity is associated with increased DNA repair competence and an induction of EMT. ALDH1A1+ cells express high level of hypoxia inducible factor-1α (HIF-1α). The expression of ALDH1A1 is regulated by the Wnt pathway through β-catenin/TCF-dependent transcription and co-occurs with the expression of β-catenin (112).

### 4. Microenvironment-mediated self-renewal of CCSCs

The tumor micro-environment represents non-tumor cells and adjacent tissues, including uncontrollable inflammation. The micro-environment experiences pathological change and provides cancer stem cells with soil, contributing to the complexity of CRC biology. The interaction between CCSCs and the micro-environment plays a role in regulating CCSC self-renewal.

**Cancer-associated fibroblasts (CAFs).** Fibroblasts in the tumor micro-environment are termed CAFs. This cell population is not stationary, but can be transformed from mesenchymal cells, endothelial cells, adipose cells and even cancer epithelial cells. CAFs promote tumor progression and invasion by both mechanical forces and metabolic machinery (113). On the one hand, CAFs induce protease-mediated extracellular matrix remodeling, serving to guide the structures that direct migration (114). On the other hand, CAFs secrete cytokines and growth factors to affect the proliferation, survival, adhesion and migration of cancer cells. The secreted mitogenic factors involve hepatocyte growth factor, EGF family members, chemokine ligand 12, fibroblast growth factors and stanniocalcin-1 (115). CAFs promote invasion. One of the mechanisms is the secretion of matrix metalloproteases or cytokines, such as tumor necrosis factor-α (TNF-α) that promotes EMT. Activated CAFs by TGF-β can trigger GP130/STAT3 signaling to express IL-11, contributing to CRC metastasis (116).

**HIF.** Anoxia is recognized to promote tumor survival and progression by inducing changes in tumor metabolism, angiogenesis, invasion, metastasis and drug resistance to reduce the clinical prognosis (117,118). Anoxia regulates gene expression through the transcriptional control of HIF-1α and HIF-2α, which can bind to hypoxia response elements (HRE) in the promoters of many genes. The induction of these genes triggers aggressive tumor growth, invasion and metastasis (117,118). HIFs are involved in the canonical Wnt pathway. HIF-1α can increase the transcriptional activity of β-catenin (119). Moreover, Newton et al found that anoxia could inhibit APC expression through the suppression of the HRE in the APC promoter by HIF-1α (120). Shay et al treated colitis-associated colon cancer with acriflavine, an inhibitor of HIF transcription, and found that it could inhibit the hypoxic induction of M-CSFR and angiogenic factors to inhibit tumor growth and progression (121).

**Myeloid-derived suppressor cells (MDSCs).** MDSCs are a heterogeneous cell population derived from bone marrow, including immature neutrophils, immature dendritic cells, immature monocytes and early myeloid progenitors (122).
recruitment from the circulation to the intestinal mucosa is associated with CXCR2 expression on CRC endothelial cells and immune cells (123). With stimulation by cytokines from the tumor stroma, such as cyclooxygenase 2 and colony-stimulating factor, MDSCs expand and generate arginase 1, ROS, and inducible nitric oxide synthase to inhibit the anticancer function of NK cells and T cells. This process contributes to the escape from immunological surveillance and promotes tumor initiation and progression (123-125). Additionally, MDSCs can secrete vascular endothelial growth factor A and matrix metalloproteinase (MMP)9 to promote angiogenesis (126). Evidence from ovarian cancer suggests that MDSCs can drive the expression of miRNA101 and subsequently suppress repressor gene CtBP2. CtBP2 interacts with nuclear genes, leading to increased stemness, tumorigenesis and metastasis in cancer cells (127).

5. Inflammation factors

Inflammatory mediators and inflammatory effectors are both important components of the tumor micro-environment. They can be derived from the intrinsic pathway, such as the mutation of an oncogene and chromosomal rearrangement, or the extrinsic pathway, such as infection (128). The two pathways activate transcription factors, mainly NF-κB and STAT3, to elicit the release of downstream inflammatory factors, such as IL-1β, IL-6, IL-8, IL-17, IL-23 and TNF-α (128,129). The inflammatory response has a series of tumor facilitating effects, including tumor initiation, survival, malignant transformation, invasion, metastasis, destruction of the adaptive immune response, influencing immune surveillance, and changing chemotherapy resistance (128,129). Activated STAT3 by IL-6 plays an important role in the early stage of colitis-associated CRC. STAT3 upregulates the expression of cell cycle regulators cyclin D1, cyclin D2, cyclin B, proto-oncogene myc, and anti-apoptotic genes Bcl-2 and Bcl-2-like 1 to promote proliferation and survival (130). IL-23 can promote CRC cells to generate TGF-β and induce the process of EMT to facilitate malignant transformation (131). Activated STAT5 by IL-23 with impaired Socs3 expression is associated with the metastasis of CRC (132), and the function of the IL-23/IL-17 axis on CRC initiation and progression has been recently recognized (133). IL-17A promotes the malignant progression of CRC through the activation of ERK, p38 MAPK and NF-κB signaling while it also regulates the production of IL-6 (134). Apart from activating multiple signaling pathways to activate transcription factors, such as STAT3 and β-catenin to regulate stemness (135), IL-8 is related to EMT. In the induction of EMT by Snail, Snail and IL-8 can form a positive feedback loop and increase the expression of stemness genes Sox2, Nanog and Oct4 (65). Leukotrienes D4 and prostaglandin E2 can increase the ALDH1 subpopulation and enhance sphere forming and tumor growth (136). The loss of TGF-β can induce the secretion of chemokine CCL9 (CCL15 in humans), which recruits immature myeloid cells that express the CCL9 receptor CCL1. These cells secrete MMP9 and MMP2 to promote CRC invasion and metastasis (137).

The components of the micro-environment are very complex. In addition to the above mentioned, others, such as blood vessels, microorganisms and normal cells, all exert an influence on the micro-environment (113,138). All of these complex components interact with each other to regulate CCSC self-renewal.

**Prospectives for CCSC-targeted intervention.** Because CCSCs possess a strong resistance to therapy, the clinical effects on CRC are very limited. The reactivation of signaling cascades, enhancement of DNA repair and drug efflux by ABC transportation may be responsible for the resistance (139). Considering that the activation of Wnt is a dominating process in the evolution and progression of CRC (26), the targeted intervention of this pathway should be most reliable. How to block this signaling pathway efficiently and specifically while maintaining normal somatic function is the aim of future study. Additionally, targets on other signaling pathways and micro-environmental factors should be included to achieve the best therapeutic effects.

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**References**

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. CA Cancer J Clin 65: 87-108, 2015.
2. De Sousa E Melo F, Vermeulen L, Fessler E and Medema JP: Cancer heterogeneity - a multifaced view. EMBO Rep 14: 686-695, 2013.
3. Zeuner A, Todaro M, Stassi G and De Maria R: Colorectal cancer stem cells: From the crypt to the clinic. Cell Stem Cell 15: 692-705, 2014.
4. Vaiopoulos AG, Kostakis ID, Koutsilieris M and Papavassiliou AG: Colorectal cancer stem cells. Stem Cells 30: 363-371, 2012.
5. Pardal R, Clarke MF and Morrison SJ: Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 3: 895-902, 2003.
6. Takahashi K and Yamanaka S: Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 126: 663-676, 2006.
7. Barker N, Ridgway RA, van Es JH, van de Wetering M, Betaghe H, van den Born M, Danenberg E, Clarke AR, Sansom OJ and Clevers H: Crypt stem cells as the cells-of-origin of intestinal cancer. Nature 457: 608-611, 2009.
8. Schepers AG, Snippert HJ, Stange DE, van den Born M, van Es JH, van de Wetering M and Clevers H: Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas. Science 337: 730-735, 2012.
9. Huang J, Papadopoulos N, McKinley AJ, Farrington SM, Curtis LJ, Wylie AH, Zheng S, Willson JK, Markowitz SD, Morin P, et al: APC mutations in colorectal tumors with mismatch repair deficiency. Proc Natl Acad Sci USA 93: 9049-9054, 1996.
10. Castets M, Brouet L, Molin Y, Brevet M, Chazot G, Gadot N, Paquet A, Mazelin L, Jarrosson-Wuilleme L, Scoazec JY, Castets M, Broutier L, Molin Y, Brevet M, Chazot G, Gadot N, Paquet A, Mazelin L, Jarrosson-Wuilleme L, Scoazec JY, et al: Intestinal tumorigenesis initiated by dedifferentiation and acquisition of stem-cell-like properties. Cell 152: 25-38, 2013.
11. Zeisel C, Eaton EN, Li SH, Chaffer CL, Reinhardt F, Kah KJ, Bell G, Guo W, Rubin J, Richardson AL, et al: Paracrine and autocrine signals induce and maintain mesenchymal and stem cell states in the breast. Cell 145: 926-940, 2011.
13. Chaffer CL, Brueckmann I, Scheel C, Kaestli AJ, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyak K, et al: Normal and neoplastic nontumor stem cells can spontaneously convert to each other. Proc Natl Acad Sci USA 106: 7850-7855, 2011.

14. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, Brooks M, Reinhardt F, Zhang CC, Shipitsin M, et al: The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 133: 704-715, 2008.

15. Yan KS, Chia LA, Li X, Ootanant S, Su J, Lee JY, Su N, Luo Y, Hechtman TR, Schmitt MA, et al: The intestinal stem cell markers BMI1 and Lgr5 identify two functionally distinct populations. Proc Natl Acad Sci USA 109: 466-471, 2012.

16. Yeung TM, Chia LA, Kosinski CM and Kuo CJ: Regulation of self-renewal and differentiation by the intestinal stem cell niche. Cytotechnology 53: 251-2523, 2011.

17. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C and De Maria R: Identification and expansion of human colon-cancer-initiating cells. Nature 445: 111-115, 2007.

18. O’Brien CA, Pollett A, Gallinger S and Dick JE: A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445: 106-110, 2007.

19. Yeung TM, Gandhi SC, Wildling JL, Muschel R and Bodmer WF: Cancer stem cells from colorectal-cancer-derived cell lines. Proc Natl Acad Sci USA 107: 12774-12779, 2010.

20. Xu X, Wang L, Zhang Z, Feng S, Zhao Y, Wu J, Dong Q, Mao J and Zhu Y: CD58, a novel surface marker, promotes self-renewal of tumor-initiating cells in colorectal cancer. Oncogene 34: 1520-1531, 2015.

21. Horst D, Kriegl L, Engel J, Kirchner T and Jung A: Prognostic significance of the cancer stem cell markers CD133, CD44, and CD166 in colorectal cancer. Cancer Invest 27: 844-850, 2009.

22. Dalerba P, Dylla SJ, Park IK, Liu R, Wang X, Cho RW, Hoey T, Gurney A, Huang EH, Simeone DM, et al: Phenotypic characterization of human colorectal cancer stem cells. Proc Natl Acad Sci USA 104: 10158-10163, 2007.

23. Huang EH, Hynes MJ, Zhang T, Gineskist C, Dontu G, Appelman H, Fields JZ, Wicha MS and Boman BM: Aldehyde dehydrogenase 1 is a marker for normal and malignant human colonic stem cells (SC) and tracks SC overpopulation during colonic tumorigensis. Cancer Res 69: 3382-3389, 2009.

24. Miranda-Lorenzo I, Dorado J, Lonardo E, Alcalà S, Serrano AG, Clausell-Tormos J, Cioffi M, Miessi D, Vinuesa AG, Aguilera C, Nye E, De Santo A: Wnt/Ca²⁺ signaling regulates the deployment of cancer progenitor phenotype on colorectal cancer cells. Cell 111: 611-615, 2002.

25. Sikandar SS, Pate KT, Anderson S, Dizon D, Edwards RA, Bertotti A, Hoey T, Plateroti M, Esposito G, Pastò A, Serafin V, Pilotto G, Lago C, Bellio C, Trusolino L, Vermeulen L, De Sousa E Melo F, van der Heijden M, Cameron K, de Jong JH, Borovski T, Tuyman JB, Todaro M, Czer C, Rodermond H, et al: Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol 12: 468-476, 2010.

26. Folde R and Brabletz T: Wnt/beta-catenin signaling in cancer stemness and malignant behavior. Curr Opin Cell Biol 19: 150-158, 2007.

27. De A: Wnt/Ca²⁺ signaling pathway: A brief overview. Acta Biochim Biophys Sin (Shanghai) 43: 745-756, 2011.

28. MacLeod RJ, Hayes M and Pacheco I: Wnt5a secretion stimulated by the extracellular calcium-sensing receptor inhibits defective Wnt signaling in colon cancer cells. Am J Physiol Gastrointest Liver Physiol 293: G403-G411, 2007.

29. Gwak J, Cho M, Gong SJ, Won J, Kim DE, Kim YI, Lee SS, Kim M, Kim TK, Shin JG, et al: Protein-kinase-C-mediated beta-catenin phosphorylation negatively regulates the Wnt/beta-catenin pathway. J Cell Sci 119: 4702-4709, 2006.

30. Fernández-Maqueda JG, Luna-Ulloa LB, Santos-Ramos P, Cañada-Llatlán M, MC and Robles-Flores M: Protein kinase δ5eta negatively modulates canonical Wnt/β-catenin pathway activity in colon cancer cells. Proc Natl Acad Sci USA 108: 4589-4594, 2010.

31. Lee JM, Kim IS, Kim H, Lee JS, Kim HY, Jeong J, Kim JH, Kim JY, Lee H, et al: RORalpha attenuates Wnt/beta-catenin signaling by PKCα-dependent phosphorylation in colon cancer. Mol Cell 37: 183-195, 2010.

32. Ishihani T, Kishida S, Hyodo-Miura J, Ueno N, Yasuda J, Waterman M, Shibuya H, Moon RT, Ninomiya-Tsuj I and Matsumoto K: The TAK1-NLK mitogen-activated protein kinase cascade functions in the Wnt5a/Ca²⁺(+) pathway to antagonize Wnt/beta-catenin signaling. Mol Cell Biol 23: 131-139, 2003.

33. Katoh M: WNT/PCP signaling in epithelial-mesenchymal transition. Curr Opin Cell Biol 18: 134-139, 2006.

34. Yeung TM, Chia LA, Kosinski CM and Kuo CJ: Regulation of beta-catenin phosphorylation/ degradation by a dual-kinase mechanism. Cell 108: 837-847, 2002.

35. Yeung TM, Chia LA, Kosinski CM and Kuo CJ: Regulation of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108: 837-847, 2002.

36.両安定 M, 他: YAP/TAZ regulation of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108: 837-847, 2002.

37. Yeung TM, Chia LA, Kosinski CM and Kuo CJ: Regulation of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. Cell 108: 837-847, 2002.
57. Kamato D, Burch ML, Piva TJ, Rezaei HB, Rostam MA, Xu S, Zheng W, Little PJ and Osman N: Transforming growth factor-β signalling: Role and consequences of Smad linker region phosphorylation. Gut 25: 2017-2025, 2017.

58. Yu M, Trobridge P, Wang Y, Kanngurn S, Morris SM, Knoblaugh S and Grady WM: Inactivation of TGF-β signalling and loss of PTEN cooperate to induce colon cancer in vivo. Oncogene 33: 1538-1547, 2014.

59. Villanueva A, García C, Paules AB, Vicente M, Megías M, Reynes G, de la Riva P, Agell N, Lluís F, Bachs O, et al: Disruption of the antiproliferative TGF-beta signaling pathways in human pancreatic cancer cells. Oncogene 17: 1969-1978, 1998.

60. Xu X, Brodie SG, Yang X, Im YH, Parks WT, Chen L, Zhou YX, Weinstein M, Kim SJ and Deng CX: Haploid loss of the tumor suppressor Smad4/Dpc4 initiates gastric polyposis and cancer in mice. Oncogene 19: 1868-1874, 2000.

61. Grady WM, Myorolf LL, Swinler SE, Rajput A, Thiagalingam S, Lutterbaugh JD, Neumann A, Brattain MG, Chang J, Kim SJ, et al: Mutual inactivation of transforming growth factor beta receptor type II in microsatellite stable colon cancers. Cancer Res 59: 320-324, 1999.

62. Woodford-Richens KL, Rowan AJ, Gorman P, Halford S, Bicknell DC, Wasan HS, Roylance RR, Bodmer WF and Tomlinson IP: SMAD4 mutations in colorectal cancer probably occur before chromosomal instability, but after divergence of the microsatellite instability pathway. Proc Natl Acad Sci USA 98: 9719-9723, 2001.

63. Zubeldia IG, Bleau AM, Redrado M, Serrano A, Gil-Puig C, Vidal-Vanaclocha F, Lecanda J and Calvo A: Epithelial to mesenchymal transition and cancer stem cell phenotypes leading to liver metastasis are abrogated by the novel TGFβ1-targeting peptides P17 and P144. Exp Cell Res 319: 12-22, 2013.

64. Moustakas A and Heldin CH: Signaling networks guiding commitment of stem cell properties in colon cancer cells. Mol Cell 45: 413-419, 2012.

65. Song J, Zhang J, Wang J, Wang J, Guo X and Dong W: β1 integrin mediates colorectal cancer cell proliferation and migration through regulation of the Hedgehog pathway. Tumour Biol 36: 1169-1177, 2015.

66. Akintoba B, Rádio-Barnett L, Barbati T, Timár J, Tóth A, et al: Analysis of CD44 and CD133 as cancer stem cell markers for colorectal cancer. Clin Cancer Res 14: 1377-1385, 2008.

67. Whisell G, Montag M, Benvenuto S, Ferreira D, Ogdon R, et al: The transcription factor GATA-4: A key regulator of colon adenoma stem cells by repressing BMP gene expression. Nat Cell Biol 16: 905-914, 2014.

68. Park Y, Kim S, Hwang S, Lee J, Park J, et al: Identification of a novel CD44 fragment that mediates CD44 activation in colorectal cancer. Oncogene 28: 2423-2431, 2009.

69. Akiyoshi T, Nakamura M, Koga K, Nakashima H, Yato T, Tsumeyoshi M, Tanaka M and Katoano M: Gli1, downregulated in colorectal cancer, inhibits proliferation of colon cancer cells involving Wnt signalling activation. Gut 55: 991-999, 2006.

70. Ariyama S, Matsunaga A, Kitamura T, Aoki K, Aoki M and Taketo MM: Reduced level of smoeduced suppresses intestinal tumorigenesis by downregulation of Wnt signalling. Gastroenterology 130: 629-638, 2006.

71. Sakamoto K and Maeda S: Targeting NF-kappaB for colorectal cancer. Expert Opin Ther Targets 14: 593-601, 2010.

72. Vlantis K, Wullaert A, Sasaki Y, Schmidt-Supprian M, Rajewsky K, Roskams T and Pasparakis M: Constitutive IKK2 activation in intestinal epithelial cells induces intestinal tumors in mice. J Clin Invest 121: 2781-2793, 2011.

73. Feng Y, Dai X, Li X, Wang H, Liu J, Zhang J, Zhu D, Yu X and Xia L: EGFR signalling pathway regulates colon cancer stem cell proliferation and apoptosis. Cell Prog 45: 413-419, 2012.

74. Al Moustafa AE, Achkar A and Yasmeen A: EGFR-receptor signaling and epithelial-mesenchymal transition in human colorectal cancers. Front Biosci (Schol Ed) 4: 671-684, 2012.

75. Munshi A and Ramesh R: Mitogen-activated protein kinases and their role in radiation response. Genes Cancer 4: 401-408, 2013.

76. Moschetta M, Reale A, Marasco C, Vacca A and Carratù MR: Therapeutic targeting of the mTOR-signalling pathway in cancer: Benefits and limitations. Br J Pharmacol 171: 3801-3813, 2014.

77. Vivancos I, Chen ZC, Tanos B, Oldrini B, Hsieh WY, Yannuzzi N, Campos C and Mellinghoff IK: A kinase-independent function of AKT promotes cancer stem cell survival, eLife 3: 3, 2014.

78. Tian Q, He XC, Hood L and Li L: Bridging the BMP and Wnt pathways by PI3 kinase/Akt and H3-3-zeta. Cell Cycle 4: 215-216, 2005.

79. Lim SC: CD24 and human carcinoma: Tumor biological aspects. Biomed Pharmacother (Suppl 2): S351-S354, 2005.

80. Ke J, Wu X, Wu X, He X, Lin L, Zou Y, He X, Wang H, Luo Y, Wang L, et al: A subpopulation of colon cancer cell lines possess stem cell characteristics. Neoplasma 59: 282-288, 2012.

81. Shluewitz M, Soloviev I, Wu T, Koeppen H, Polakis P and Sakanaka C: Repressor roles for TCF-4 and Sfrp1 in Wnt signalling in breast cancer. Oncogene 25: 4361-4369, 2006.

82. Muppala S, Mudduluru G, Leupold JH, Byerly D, Sleeman JP and Allgayer H: CD24 induces expression of the oncomir miR-21 via Src, and CD24 and Src are both post-transcriptionally downregulated by the tumor suppressor miR-34a. PLoS One 8:e59563, 2013.

83. Wang YC, Wang JL, Kong X, Sun TT, Chen HY, Hong J and Fang JY: CD24 mediates gastric carcinogenesis and promotes gastric cancer progression via STAT3 activation. Apmis 19: 643-656, 2014.

84. Simms LA, Pike T, Young JP, Jass JR, et al: Evaluation of CD44 and CD133 as cancer stem cell markers for colorectal cancer. Cancer Res 65: 4645-4652, 2005.

85. Fujita S, Watanabe M, Kubota T, Teramoto T and Kitajima M: Alteration of expression in integrin beta 1-subunit correlates with invasion and metastasis in colorectal cancer. Cancer Lett 91: 145-149, 1995.

86. Song J, Zhang J, Wang J, Wang J, Guo X and Dong W: β1 integrin mediates colorectal cancer cell proliferation and migration through regulation of the Hedgehog pathway. Tumour Biol 36: 2013-2021, 2015.

87. Marhaba R and Zöller M: CD44 in cancer progression: Adhesion, migration and growth regulation. J Mol Histol 35: 211-231, 2004.

88. Seyle EC, Jurado GA, Brunson BA, Wakefield JK, Frost AR and Bellis SL: Hypersialylation of betal integrins, observed in colon adenocarcinoma, may contribute to cancer progression by upregulating cell motility. Cancer Res 65: 4645-4652, 2005.

89. Tóth A, Teramoto T, Kubota T and Kitajima M: Evaluation of CD44 and CD133 as cancer stem cell markers for colorectal cancer. Clin Cancer Res 14: 6751-6760, 2008.

90. Bánky B, Rádió-Barnett L, Barbati T, Timár J, Becsíg P and Rásó E: Characteristics of CD44 alternative splice pattern in the course of human colorectal adenocarcinoma progression. Mol Cancer 9: 179, 2010.

91. Wielenga V, Smits R, Korinek V, Smit L, Kiemen M, Fodde R, Clevers H and Pals ST: Expression of CD44 in Apc mutant mice implies regulation by the Wnt pathway. Am J Pathol 154: 515-523, 1999.

92. Wang C, Xie J, Guo J, Manning HC, Gore JC and Guo N: Evaluation of CD44 and CD133 as cancer stem cell markers for colorectal cancer. Oncol Rep 28: 1301-1308, 2012.

93. Schmitt M, Metzger M, Gradl D, Davidson G and Orian-Rousseau V: CD44 functions in Wnt signaling by regulating LRPs and CD44 and CD133 are functional importance for colorectal cancer stem cells. Clin Cancer Res 14: 6751-6760, 2008.
McIntyre A and Harris AL: Metabolic and hypoxic adaptation
2012.

117.

116.

115.

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141. May R, Riehl TE, Hunt C, Sureban SM, Anant S and Houchen CW: Identification of a novel putative gastrointestinal stem cell and adenoma stem cell marker, doublecortin and CaM kinase-like-1, following radiation injury and in adenomatous polyposis coli/multiple intestinal neoplasia mice. Stem Cells 26: 630-637, 2008.

142. Li D, Peng X, Yan D, Tang H, Huang F, Yang Y and Peng Z: Msi-1 is a predictor of survival and a novel therapeutic target in colon cancer. Ann Surg Oncol 18: 2074-2083, 2011.

143. Yu T, Chen X, Zhang W, Colon D, Shi J, Napier D, Rychahou P, Lu W, Lee EY, Weiss HL, et al: Regulation of the potential marker for intestinal cells, Bmi1, by β-catenin and the zinc finger protein KLF4: Implications for colon cancer. J Biol Chem 287: 3760-3768, 2012.

144. Sagiv E, Memeo L, Karin A, Kazanov D, Jacob-Hirsch J, Mansukhani M, Reichav G, Hibshoosh H and Arber N: CD24 is a new oncogene, early at the multistep process of colorectal cancer carcinogenesis. Gastroenterology 131: 630-639, 2006.

145. Haraguchi N, Ishii H, Mimori K, Ohta K, Uemura M, Nishimura J, Hata T, Takemasa I, Mizushima T, Yamamoto H, et al: CD49f-positive cell population efficiently enriches colon cancer-initiating cells. Int J Oncol 43: 425-430, 2013.

146. Gemi P, Mirabelli P, Di Noto R, Corbo C, Iaccarino A, Zamboli A, Troncone G, Galizia G, Lieto E, Del Vecchio L, et al: CD66c is a novel marker for colorectal cancer stem cell isolation, and its silencing halts tumor growth in vivo. Cancer 119: 729-738, 2013.

147. Nakanishi Y, Seno H, Fukuoka A, Ueo T, Yamaga Y, Maruno T, Nakanishi N, Kanda K, Komekado H, Kawada M, et al: Dlk1 distinguishes between tumor and normal stem cells in the intestine. Nat Genet 45: 98-103, 2013.

148. van der Flier LG, Haegebarth A, Stange DE, van de Wetering M and Clevers H: OLFM4 is a robust marker for stem cells in human intestine and marks a subset of colorectal cancer cells. Gastroenterology 137: 15-17, 2009.