Molecular insights into colorectal cancer stem cell regulation by environmental factors

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Abstract

Colorectal cancer remains a significant cause of cancer-related mortality worldwide, mainly because of tumor relapse and metastases. Cancer stem cells (CSCs) are considered to be the main cause of resistance to chemotherapeutic agents, as well as being responsible for maintaining their CSC properties. Furthermore, extensive investigations have revealed that obesity, accompanied by excess visceral adipose tissue, induces chronic inflammation, and is linked to the risk and progression of several gastrointestinal cancers, through modulating the capacities of the CSCs. This review presents the evidence linking colorectal CSCs and their environment and summarizes our current understanding of the molecular mechanisms underlying this relationship.

Key words: Cancer stem cells markers, colorectal cancer stem cell, nutrient, obesity, tumor microenvironment

Introduction

Colorectal cancer (CRC) is the fourth-leading cause of cancer-related deaths worldwide.[1] Although the incidence of CRC has started to decline in developed countries, it continues to increase in developing countries.[2] Environmental factors, including chronic inflammation, obesity, metabolism and nutrition, have become recognized as major contributors to the development of CRC.[3-6] Dietary fat intake and obesity have been shown to be significantly involved in CRC progression through an increased risk of gene mutation, epigenomic alterations, and effects on the equilibrium of various adipokines.[7-11] Chronic inflammation is also considered to be a risk factor for CRC,[12] and inflammatory mediators and substances such as interleukin (IL)-6, tumor necrosis factor-α (TNF-α), and reactive oxygen species have been shown to affect CRC development.[12-15] The clearest link between chronic inflammation and CRC is seen in patients with inflammatory bowel disease, which has been reported to promote tumorigenesis by altering the microbial composition in the gut and supporting the expansion of microorganisms with genotoxic capabilities.[16]

Cancer stem cells (CSCs) are tumor cells that possess capabilities for self-renewal, clonal tumor initiation and clonal long-term repopulation.[17,18] The discovery of colorectal CSCs highlighted the existence of intratumoral heterogeneity, revealing the presence of tumor cells expressing markers characteristic of immature cells and with increased abilities to resist chemotherapy and to seed secondary tumors.[19-21] CSCs were initially considered to be a cell population with well-defined phenotypic and molecular features. However, emerging evidence has revealed that certain cancer cells exhibit plasticity, and can change reversibly from stem to non-stem cells under the regulation of genetic, epigenetic and microenvironmental factors.[22-25] In this review, we focused on accumulating new evidence indicating that microenvironmental factors maintained colorectal CSC properties responsible for promoting tumor development and metastasis.

Markers for Colorectal CSCs

CSCs have been isolated from cancer tissues using flow cytometry with specific surface markers. Several molecules have been proposed as colorectal...
CSC markers, including CD133, CD44, CD24, CD166, Lgr-5, and aldehyde dehydrogenase 1 (ALDH1) [Table 1]. CD133, a pentaspan transmembrane glycoprotein, was one of the first colorectal CSC markers to be identified. However, although selecting CRC cells based on AC133 positivity, an epitope of the CD133 protein identifies the tumorigenic and clonogenic population. CD133 expression has been detected throughout the normal gastrointestinal tract and is not restricted to the stem cell compartment. In addition, both CD133+ and CD133- metastatic CRC cells were able to form new tumors, suggesting that CD133 may not be a reliable marker of CSCs.

The cell adhesion molecule CD44 has been identified as a cell surface marker associated with CSCs in several types of tumor. CD44+ cells exhibited CSC properties, and a single cell could form a sphere in vitro, and a xenograft tumor resembling the original lesion in vitro. Overexpression of CD44 in CRC has been associated with depth of invasion and lymph node involvement and is shown to be an independent predictor of overall survival. Although CD44, like CD133, is not a specific marker for colorectal CSCs, it is possible that a combination of these two markers may be more reliable for detecting colorectal CSCs than either marker alone.

In addition to cell surface markers, activities of certain pathways or enzymes may also act as markers of stemness. For instance, normal colorectal stem cells can be identified by the activity of ALDH1, a detoxifying enzyme that oxidizes intracellular aldehydes. ALDH1+ cells were sparse and restricted to the bottom of normal crypts, where stem cells reside but were increased in number and distributed further up the crypts during progression from normal epithelium to adenoma. In addition, implantation of ALDH1+ colon cancer cells into NOD/SCID mice generated xenograft tumors, whereas ALDH1- cells did not. These findings indicate that ALDH1 activity may be a useful colorectal CSC marker.

Other markers include CD166, epithelial cell adhesion molecule, CD29, CD24, CD26, Msi-1, Lgr-5, and Wnt activity/β-catenin. The presence of these molecules has been associated with stemness characteristics both in vitro and in vivo. These markers were also used to enrich isolated CSCs further to enhance their tumorigenic ability. The transcription factors Oct-4 and Sox2 are also promising CSC markers, given their roles in cell renewal. Oct-4 and Sox2 levels have been shown to be elevated in CRC and to correlate with increased CSC proliferation and poor prognosis. Other pluripotency genes, Nanog, Lin-28, Klf-4, and c-myc, are regarded as promising surrogate markers, given that they appear to facilitate a shift towards an undifferentiated state.

Table 1: CRC stem cell markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>General function</th>
<th>Significance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD133 (Prominin-1)</td>
<td>Pentaspan transmembrane glycoprotein</td>
<td>Tumor initiation in xenografts, colony formation, correlation with: poor prognosis, survival, metastasis, resistance to therapy</td>
<td>[28-31,41,43,45]</td>
</tr>
<tr>
<td>CD44</td>
<td>Cell adhesion molecule, hyaluronic acid receptor</td>
<td>Tumor initiation in xenografts, colony formation, association with tumor stage, lymph node infiltration, survival</td>
<td>[32-36,41,43,45]</td>
</tr>
<tr>
<td>ALDH1-</td>
<td>Detoxifying enzyme</td>
<td>Tumor initiation in xenografts, further enrichment, transition from colitis to cancer, mitochondrial isofrom is increased in CRC</td>
<td>[37-39,41]</td>
</tr>
<tr>
<td>CD166 (ALCAM)</td>
<td>Cell adhesion molecule</td>
<td>Tumor initiation in xenografts, colony formation, further enrichment, correlation with prognosis and survival</td>
<td>[41,45]</td>
</tr>
<tr>
<td>EpCAM</td>
<td>Cell adhesion molecule</td>
<td>Expression in CD133+ or CD44+ cells</td>
<td>[41]</td>
</tr>
<tr>
<td>CD29 (β1-integrin)</td>
<td>Receptor for ECM</td>
<td>Colony formation elevated in CRC, association with tumor stage</td>
<td>[41,45]</td>
</tr>
<tr>
<td>CD24</td>
<td>Cell adhesion molecule</td>
<td>Clonogenic ability, multilineage potential, further enrichment, correlation with invasiveness, differentiation, and survival</td>
<td>[41,45]</td>
</tr>
<tr>
<td>CD26</td>
<td>Cell surface glycoprotein</td>
<td>Tumor initiation and metastasis formation in a mouse model</td>
<td>[43]</td>
</tr>
<tr>
<td>Msi-1</td>
<td>Maintenance of the undifferentiated state</td>
<td>Expression in CD133+ cells and spheroid cultures, association with tumor stage</td>
<td>[22]</td>
</tr>
<tr>
<td>Lgr-5</td>
<td>Wnt target gene, crypt base restriction</td>
<td>Tumorigenicity, poor prognostic factor, metastasis formation, adenoma development in APC knockout mice</td>
<td>[40-42,44,45]</td>
</tr>
<tr>
<td>Wnt activity/β-catenin</td>
<td>Maintenance and proliferation of the SC reservoir</td>
<td>Associated with clonogenicity and tumorigenicity, detection of low stage CRC cases with high risk of relapse</td>
<td>[40-42,44,45]</td>
</tr>
<tr>
<td>Oct-4, Sox2, Nanog, Lin-28, Klf-4, c-Myc</td>
<td>Transcription factors</td>
<td>Correlation with poor prognosis, relapse, distant recurrence, resistance to therapy</td>
<td>[46-48]</td>
</tr>
</tbody>
</table>

ALDH1: Aldehyde dehydrogenase-1; CRC: Colorectal cancer; ALCAM: Activated leukocyte cell adhesion molecule; EpCAM: Epithelial cell adhesion molecule; ECM: Extracellular matrix; Lgr-5: Leucine-rich repeat containing G protein-coupled receptor 5; Msi-1: Musashi-1; SC: Stem cell; APS: Adenomatous polyposis coli
Colorectal CSCs Niche in the Tumor Microenvironment

Tissue stem cells reside in their surrounding microenvironment, known as the stem cell niche, and play an essential role in maintaining tissue homeostasis through their abilities of self-renewal and differentiation. Lgr5+ stem cells in the intestinal crypts are interspersed among terminally differentiated Paneth cells, which act as guardians of the stem cells by providing essential niche signals. The tumor microenvironment surrounding cancer cells contains multiple cell types including immune cells, endothelial cells, and fibroblasts, in addition to the extracellular matrix. Recent evidence suggests that cancer cells interact with their microenvironment and each other by secreting growth factors, cytokines, and proteases. Furthermore, the properties of the CSCs depend on the CSC niche, which regulates their proliferation and differentiation, as well as those of the tissue stem cells.

Mesenchymal stem cells (MSCs) have been shown to be recruited into the tumor stroma, and to enhance tumor growth and metastasis in CRC. MSCs are considered as potential precursors of carcinoma-associated fibroblasts (CAFs, also known as tumor-associated fibroblasts), which play a key role in tumor progression in various types of cancer, including CRC. Carcinoma-cell-derived IL-1 was shown to induce prostaglandin E2 (PGE2) secretion by MSCs, and the resulting PGE2 then acted in an autocrine manner with ongoing paracrine IL-1 signaling to induce expression of cytokines by the MSC, thus creating a CSC niche. A recent study demonstrated that CRC cells can induce adjoining bone-marrow-derived MSCs to exhibit the typical characteristics of CAFs in vitro, and activated Notch signaling mediates transformation of bone-marrow-derived MSCs to CAFs through the downstream TGF-β/Smad signaling pathway. Cytokines secreted by CAFs, including hepatocyte growth factor, osteopontin, and stromal-derived factor 1α, increase CD44v6 expression in colorectal CRCs, which in turn promote migration and metastasis. Another study demonstrated that CSCs were resistant to conventional chemotherapy and that chemoresistance was also increased by CAFs. In this study, chemotherapy-treated human CAFs promoted CSC self-renewal and in vivo tumor growth associated with secretion of cytokines and chemokines, including IL-17A.

The Wnt/β-catenin signaling pathway has been shown to play critical roles during the transition from normal colorectal mucosa to adenocarcinoma. The tumor microenvironment may play a central role in malignant transformation by locally modifying β-catenin activity in tumor cells, thus contributing to tumor growth and cancer stemness. Likewise, myofibroblast-secreted factors, especially hepatocyte growth factor, activated Wnt signaling and restored the CSC phenotype in more differentiated tumor cells both in vitro and in vivo.

Several studies have reported that CSCs reside in perivascular niches in certain types of cancer. Endothelial-cell-derived, soluble Jagged-1 led to Notch activation in colorectal CSC cells in a paracrine manner, thus promoting the CSC phenotype. Hypoxia is known to play pivotal roles in cell survival, angiogenesis, tumor invasion and metastasis, and is involved in the maintenance of self-renewal and the undifferentiated state of CSCs in various types of tumors. According to a study of colorectal cell line-derived CSCs, hypoxia maintained their stem-like phenotype and prevented differentiation of enterocytes and goblet cells by regulating CDX1 and Notch1.

Obesity, Nutrients, and Colorectal CSCs Properties

Obesity and visceral adiposity are closely related to disorders such as diabetes, cardiovascular disease, and increased risk of various cancers, including CRC. Although a meta-analysis showed that an increase in the body mass index in men was associated with a relative CRC risk of 1.24, the relationship between increased body mass index and CRC risk in women is inconsistent. It is possible that the insulin and the insulin-like growth factor-1 axis may play different roles in colorectal carcinogenesis in men and women.

Visceral obesity is associated with increased infiltration of inflammatory cells such as macrophages and T-cells into the adipose tissue, together with low-grade inflammation. Adipose tissues produce various growth factors, hormones, and cytokines known as adipocytokines, including leptin, resistin, visfatin, adiponectin, and numerous inflammatory mediators such as TNF-α, IL-6, IL-8, IL-10, and IL-1 receptor agonists. These adipose-derived factors have demonstrated an intimate involvement in increased risk of CRC. In addition to adipocytokine-mediated inflammation, dyslipidemia, insulin resistance, and activation of the renin-angiotensin system may also contribute to CRC development.

Colorectal CSC clones have been reported to express leptin receptors and to respond to leptin by cell proliferation, activation of the ERK1/2 and PI3K/AKT signaling pathways, enhanced growth in soft agar, and improved sphere formation associated with E-cadherin overexpression. Moreover, leptin counteracted the cytotoxic effects of 5-fluorouracil. Other authors reported that leptin acted as a growth factor for carcinogen-induced colorectal tumors in a mouse model of obesity. They also showed that leptin receptor expression levels were markedly increased in colorectal tumors compared with normal epithelium, in association with activation of Wnt signaling.

Chronic inflammation is considered to be a risk factor for CRC, and an obvious association has been demonstrated between the incidence of CRC and inflammatory
bowel diseases, such as ulcerative colitis and Crohn’s disease. A recent study showed that the inflammatory lipid mediators leukotriene D4 and PGE2 increased the ALDH+ cell population, colony formation capacity, and tumor growth in a xenograft model of colon cancer. A high-fat diet can cause changes in the composition of the intestinal microbiota, and affect gut immune and inflammatory effectors implicated in intestinal tumorigenesis. In contrast, omega-3 polyunsaturated fatty acids (PUFAs) have shown substantial benefits in patients with the chronic inflammatory disease. In a placebo-controlled, randomized controlled trial, administration of omega-3 PUFAs decreased polyp number, size, and overall burden in patients with familial adenomatous polyposis. Omega-3 PUFAs were shown to inhibit proliferation and angiogenesis, and exert a pro-apoptotic effect in several in vitro models of CRC. One possible molecular mechanism involves the G-protein-coupled receptor 120, which functions as an omega-3 fatty acid receptor/sensor in pro-inflammatory macrophages and mature adipocytes and represses the production of TNF and IL-6, as well as macrophage-induced tissue inflammation. Furthermore, omega-3 PUFAs down-regulated the expression of CRC stem-like cell marker CD133, and up-regulated the colorectal epithelium differentiation markers cytokeratin 20 and mucin 2. A recent study revealed that the low-cytotoxic combination of eicosapentaenoic acid-free fatty acid, epigallocatechin-3-gallate, and grape-seed extract (GSE) inhibited mammalian target of rapamycin signaling and thus reduced cell proliferation and induced apoptosis in CRC cells. GSE pre-treatment of adipocytes decreased their growth-promoting effects on CRC cells. In addition, adipocyte-conditioned media collected after chronic and acute pre-treatment with GSE significantly reduced the chemotactic properties of adipocytes toward CRC cell invasion. Finally, GSE decreased the expression of CD44 and inhibited adipocyte-mediated pro-tumorigenic signals in CSC-enriched colonospheres. Overall, these findings indicate a close link between obesity and chronic inflammation, leading to CRC progression through enhanced colorectal CSC properties, whereas some nutrients decrease the expression of CSC markers and attenuate the properties of CSCs.

Conclusion

The microenvironment surrounding cancer cells forms the CSC niche, allowing them to give rise to a hierarchy of proliferative and differentiating cells. Targeting the innate pathways and molecules between colorectal CSCs and their environment may thus represent a promising therapeutic strategy, and may provide a complementary approach to conventional therapies that target the malignant cells themselves. Anti-tumorigenic agents related to nutrients in the microenvironment may have particular potential to eliminate the population of colorectal CSCs. Further understanding of the molecular mechanisms underlying the regulation of CSC properties by environmental factors may lead to the development of potential therapeutic targets for patients with CRC.

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Conflicts of interest

There are no conflicts of interest.

References


