Review

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Targeting Stem Cells in the Colorectal Cancer Microenvironment to Avert Drug Resistance in Pursuit of Novel Oncotherapies

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Several studies have demonstrated the heightened prevalence of colorectal cancer (CRC) among young black men. Most of these men present with already metastasized CRC. Cancer stem cells (CSCs) play a pivotal role in CRC metastasis and drug resistance. The plasticity of CSCs promotes therapeutic resistance by continuously dividing into different phenotypes thwarting therapeutic targets. Phenotypic changes affect the expression of highly heterogeneous surface biomarkers. Identifying molecular and cell surface biomarkers is important for diagnosis, decision-making, and determining clinical outcomes. Furthermore, CSCs promote cancer initiation, development, advancement, relapse, and therapeutic resistance by altering the tumor microenvironment (TME). Cancer-favoring molecular signaling pathways may contribute to differentiating CSCs into TME components that create favorable conditions conducive to cancer progression. In turn, different TME components may differentially stimulate CSCs, prompting proliferation into diverse cancer cell phenotypes. This review describes the mechanisms of CSCs in promoting CRC and elucidates how the TME and CSCs work synergistically to sustain cancer development, evoke relapse, and promote therapeutic resistance. These cancer-promoting mechanisms can be antagonized by identifying different CSC phenotypes and targeting them for cancer therapy.

Keywords: colorectal cancer; tumor microenvironment; cancer stem cells; drug resistance; mesenchymal stem cells; epithelial mesenchymal transition; metastasis

Introduction

The incidence of colorectal cancer (CRC) markedly rises with increasing age. However, the increase in young people (<50 years), surged by 80% to 100% per 100,000 individuals during the period 2015–2019 in the United States [1]. Young black men exhibit a notably earlier onset of CRC, with a median age of 56 compared to other racial groups at 62 years old [2]. Among patients younger than 65 years, the annual incidence rate of localized disease (2–3%) and metastatic disease (0.5–3%) is increasing since 2010 [1]. Many patients succumb to CRC, with about 20% of patients presenting with metastatic disease at first diagnosis and about 35–45% of patients relapsing after surgery [3].

Cancer stem cells (CSCs) are linked to cancer metastasis [4]. Cells isolated from colon cancer, positive for stem cell marker CD133 have been demonstrated the ability to initiate cancer when transplanted into immunodeficient Nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice [5]. Similar results were observed when injecting SW1222 cell line megacolonies into the same mouse model [6]. These CSCs possess the attributes of stemness and self-renewal capacity, tightly linked to metastatic potential, constituting a major challenge in the treatment of CRC. Moreover, CSCs have also been associated with therapeutic resistance. The characteristic features of CSCs are maintained by the physicochemical composition of the tumor microenvironment (TME), further promoting metastasis and therapeutic resistance [7]. Consequently, Yan and Su [8] proposed the classification of CRC using a CSC-based model.

The heterogeneous nature of CSCs in the TME plays an important role in therapeutic resistance [9], and a continuous supply of CSCs in the TME is important for cancer survival [10]. The TME has been shown to provide suitable conditions to facilitate the differentiation of normal stem cells into CSCs. Once CSCs are generated, they have the potential to differentiate into protumorous cells such as cancer associated fibroblasts (CAFs), tumor associated macrophages, and tumor endothelial cells (TECs) [11]. A

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process, known as angiogenesis, results in the formation of new blood vessels that supply oxygen and nutrients to the tumor. TECs (tumor endothelial cells) line these blood vessels and contribute to vasodilation and vasoconstriction, facilitating the transport of essential nutrients and waste products in the TME [12]. The ability of CSC to resist therapy and survive is orchestrated by the conducive architectural structure and biochemical components of the TME. Thus, understanding the characteristics of CSCs in TMEs is pivotal in targeting tumor development and metastasis in CRC.

While the components of the TME undeniably influence cancer initiation and progression [13], it is essential to acknowledge that the physiochemical components of the TME varies across different cancer types. Consequently, the physiochemical composition of the TME affects how different cancers respond to treatment. Myeloid cells, including tumor associated macrophages, myeloid-derived suppressor cells, dendritic cells (DCs) and, cancer circulating neutrophils, constitute the most abundant cell population in the TME. These cells primarily regulate the TME by modulating lymphocyte responses [14]. Furthermore, intracellular signaling and intercellular crosstalk in the TME serve as main facilitators of cancer progression and therapeutic resistance. Therefore, targeting this crosstalk holds therapeutic promise [15]. In light of these considerations, we discuss the interplay between CSCs and CRC development, relapse, and therapeutic resistance. We elucidate the mutual association between the TME and CSCs, which sustains favorable conditions for tumor growth, ultimately contributing to drug resistance and cancer recurrence.

Generation of CSCs from Normal Progenitors or Other Stem Cell Phenotypes

CSCs may originate from adult tissue-resident stem cells or dedifferentiated cells resulting in two types of CSCs. The first type comprises CSCs that do not express connexins or gap junction genes, resulting in their inability to engage in gap junctional intercellular communication. The second type of CSCs have dysfunctional gap junction genes that result in similar intercellular communication dysfunctionality, leading to uncontrolled tumor growth. Hence, pinpointing the specific type of CSCs may facilitate the development of precise and targeted cancer therapies [16].

Whilst Liu [17] posits that cancer originates from stem cells via dedifferentiation, occurring through a blastomeric or blastomere-like program that ceases at a specific developmental hierarchy, Luo *et al*. [18] recently described CSCs as stem cells with disordered or uncontrolled differentiation, suggesting a monophyletic model derived from the historical concept of the origin of CSCs. Liu [17] asserts that the transition between the development of malignant or benign tumors depends on the developmental hierarchy at which stem cells arrest. Conversely, Luo *et al*. [18] suggest that the degree of differentiation is genetically determined.

Afify *et al*. [19] generated CSCs by injecting induced pluripotent stem cells (iPSCs) treated with conditioned mediums from a liver cancer cell line into the livers of Bagg Albino nude mice. The cancer cells resected from these mice had self-renewal capabilities with increased expression of liver CSC markers. In a different experiment, Okita *et al*. [20] generated iPSCs by introducing Oct3/4 retroviral vectors into mouse fibroblasts. These iPSCs could be differentiated into CSCs by exposing them to conditioned medium from a mouse Lewis lung carcinoma cell line [21] (Fig. 1). While iPSCs can be successfully differentiated into various cell types for therapeutic purposes, several challenges [22] including the formation of teratomas [23] and the potential for cancer formation in various tissues hinders their clinical application [24].

Mesenchymal stem cells (MSCs) play a pivotal role in shaping the TME and its function [25]. Notably, MSCs are known to promote the formation of the CRC TME by secreting interleukin-6 (IL-6), which activates signal transducer and activator of transcription 3 (*STAT3*). Targeting *IL-6* and *STAT-3* has also shown promise in attenuating CRC tumor formation [26]. Zhang *et al*. [27] further reported that IL-6 was highly expressed in human CRC MSCs and that the expression of *IL-6* correlates with CRC progression via *IL-6*/*JAK2*/*STAT3* signaling and increased invasion and migration of CRC cells.

Cancer initiation can also be triggered via epigenetic reprogramming induced by the presence of cancer cells which promote the conversion of MSCs into protumorous cancer-associated MSCs [28]. MSCs migrate to the TME, where they emerge as important mediators of cancer progression. In the TME, MSCs transform into tumor favoring CSCs, also referred to as cancer associated MSCs and CAFs. Infiltration of MSCs into the TME is notably heightened [29], and they have been shown to increase in other regions such as the pulmonary arterial blood of patients with lung cancer [30]. These MSCs, originating in the bone marrow, exhibit immunosuppressive effects. Remarkably, MSCs can migrate back to the bone marrow, retaining their enhanced immunosuppressive potential. These cancer-associated MSCs/CSCs stimulate the development of bone marrow-derived polymorphonuclear myeloid-derived suppressor cells, which facilitate cancer progression in the TME and at metastatic sites [31] (Fig. 1). Cancer-associated MSCs promote cancer aggressiveness and therapeutic resistance through several mechanisms, including angiogenesis, recruitment of immunosuppressive myeloid cells into the TME [32] and several other immune related mechanisms [33]. These include suppressing chimeric antigen receptor T-cell mediated cytotoxicity, as evidenced by an increase in regulatory T cells, indoleamine 2,3-dioxygenase, and programmed cell death-ligand 1 in co-culture experiments with lymphoma cells and macrophages. This immunosuppressive effect was reversed when the stanniocalcin-1 (*STC1*)

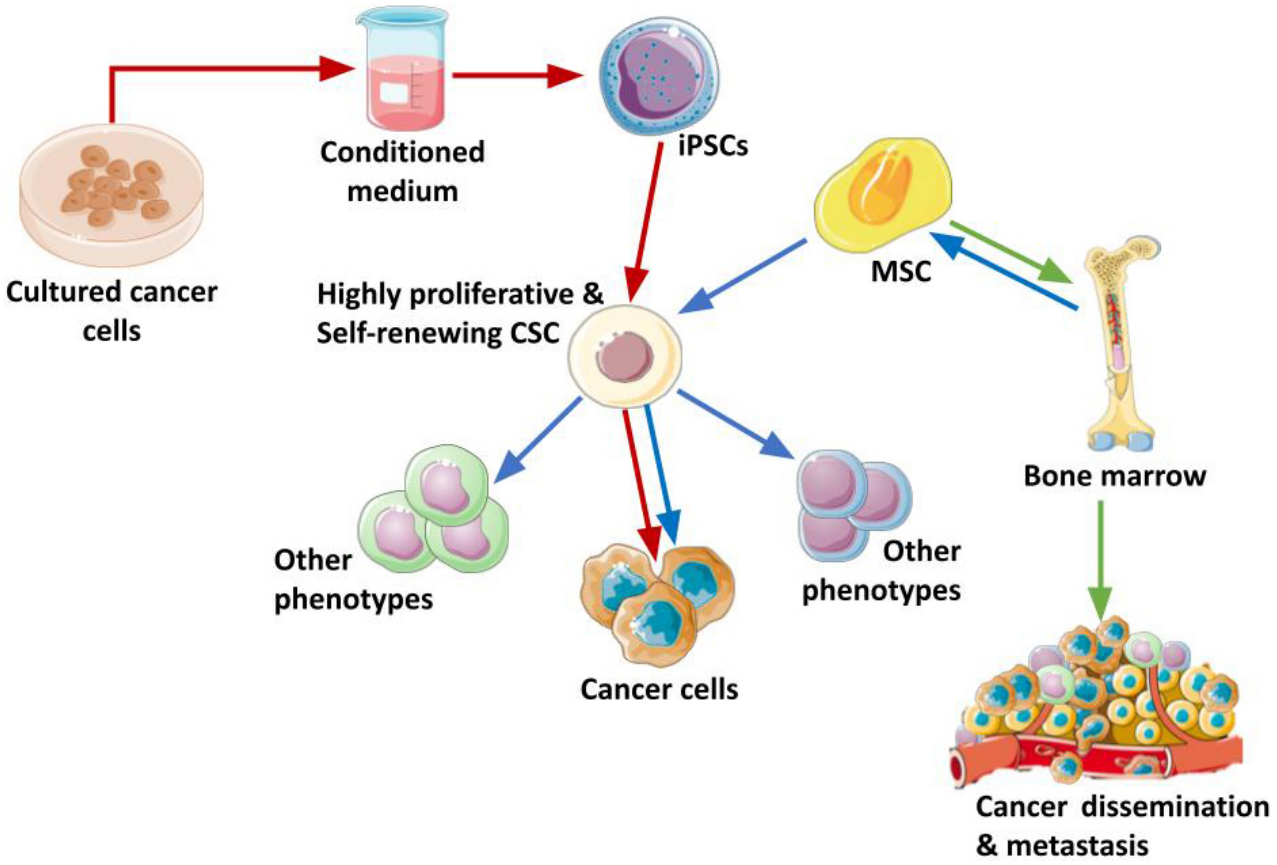

Fig. 1. Development of cancer stem cells and differentiation into heterogeneous phenotypes. CSC, cancer stem cell; iPSCs, induced pluripotent stem cells; MSC, mesenchymal stem cell.

gene in MSCs was knocked down. Consequently, MSCs, particularly CSCs and *STC1* should be considered when developing therapeutic strategies to unravel therapeutic resistance in cancer [34].

CSCs can be generated *in vitro* by exposing healthy harvested stem cells to medium derived from cancer cells. These induced pluripotent stem cells exhibit a remarkable capacity for self-renewal. Consequently, they transition into CSCs characterized by heightened proliferative abilities that contribute to cancer progression. CSCs can also be generated from bone marrow MSCs recruited to the TME. These cells manifest the same features as other CSCs with an even more immunosuppressive capacity upon their return to the bone marrow thus increasing their capacity to sustain cancer cells both in the TME and at metastatic sites.

The Physicochemical Composition of the TME in Relation to CSCs and CRC Progression

In the TME, CSC populations exhibit epithelial mesenchymal transition (EMT), with a distinct distribution. Spatially, MSCs are primarily located at the tumor periphery, while hybrid epithelial/mesenchymal (E/M) CSCs tend to occupy the central region of the tumor [35]. Several cytokines including transforming growth factor beta (TGF-β) and IL-6 induce Notch/jagged signaling which ensures the stability of the hybrid E/M phenotype. Furthermore, Notch/jagged signaling can govern both the density of CSC populations and the spatial separation between these populations [36] (Fig. 2). In the context of CRC, the Notch signaling pathway maintains CSC stemness and facilitates chemotaxis, which promotes metastasis [37]. Notch/jagged signaling further regulates cancer cells migration by either inducing or downregulating angiogenesis. The homeostatic balance between the delta-like 4 (DLL4) and jagged-1 (Jag1) proteins determines the structural integrity of the tumor vasculature. Elevated levels of Jag1 result in increased tumor vascularity, resulting in dysregulated angiogenesis. Conversely, lower levels of Jag1 have the opposite effect on tumor vascularity, promoting tumor growth [38] (Fig. 2).

Approximately 40–50% of new CRC cases metastasize. This is often attributed to the overexpression of the Notch signaling pathway [39]. Notch signaling actively interacts with other immune regulatory pathways such as NF-κB signaling to promote cancer [40]. Interestingly, this effect can be mitigated by loss-of-functional mutations in the Notch signaling pathway, particularly among patients with microsatellite instability (MSI) tumors. This potential alteration may facilitate the recruitment of anti-cancer immune cells into the CRC TME [41]. Using a Notch reporter

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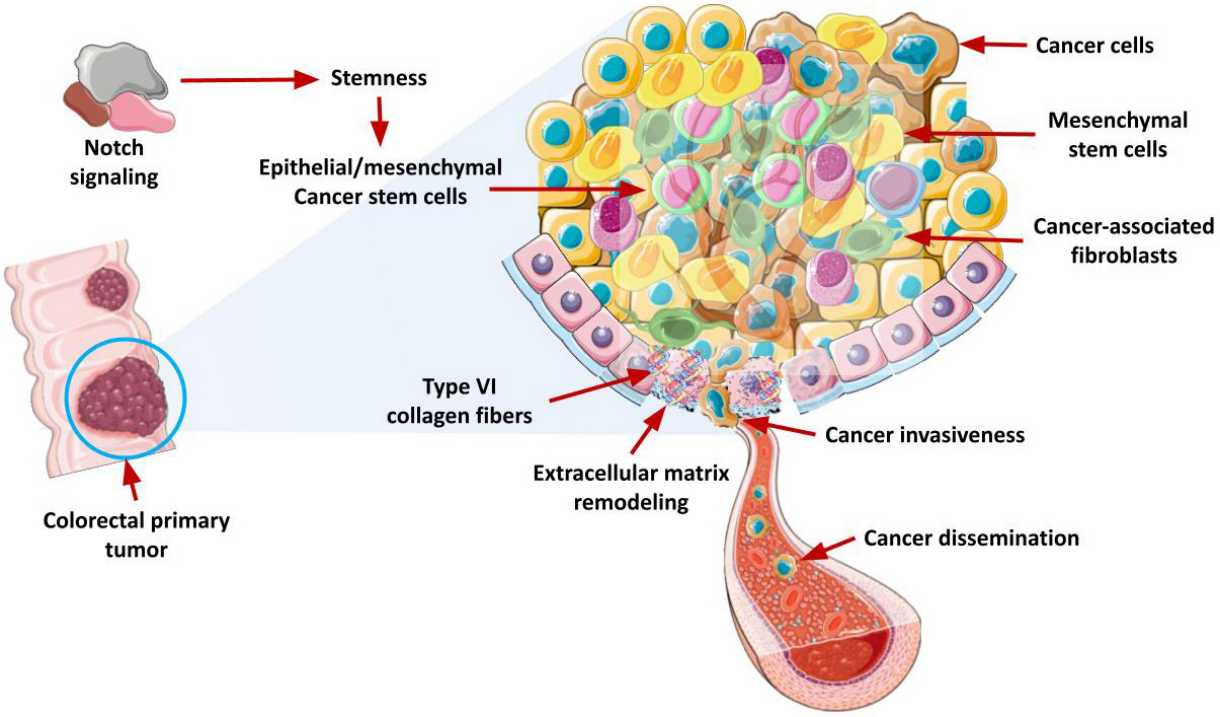

Fig. 2. The involvement of a complex tumor microenvironment in maintaining cancer stem cells and promoting cancer progression. Mesenchymal stem cells are primarily located at the edges of the tumor whilst cancer stem cells are intra-tumoral where they play a major role in ensuring the reproducibility of cancer cells, inducing epithelial mesenchymal transition, and promoting metastasis.

gene assay, Kretschmer *et al*. [42] observed persistent activation of the Notch signaling pathway on softer substrates, achieved through activation via a *DLL4* overexpressing cell line or rhDLL4 protein coating. They observed higher immunofluorescence staining intensity, highlighting nuclear localization of the Notch intracellular domain in softer substrates [42].

Matrix stiffness is important in the initiation and progression of CRC. CRC has a complex mechanical microenvironment that is subject to various stimuli, including normal colonic motility, heightened cancer cell proliferation leading to increased intra-tumoral pressure, increased collagen and extracellular matrix (ECM) deposition, reduced matrix degradation leading to ECM stiffness and upregulation of CAFs, all of which result in enhanced endogenous stress. CAFs play an important role in ECM stiffness [43] (Fig. 2). CAFs are known to contribute to CRC progression, invasion, metastasis, and therapeutic resistance through ECM remodeling [44–46]. The ECM is primarily made up of collagen, with type V collagen being implicated in basement membrane breakdown and promoting oncogenesis, especially when compared to collagens found within the stroma [47]. CAFs in the CRC microenvironment have been shown to exhibit elevated levels of type VI collagen (Fig. 2). Human fibroblasts, induced by twist related protein 1 (*Twist1*), acquire a CAF phenotype leading to increased matrix stiffness. Notably, palladin and type VI collagen have been identified as mediators of Twist1-induced CAFs. These two elements are markedly expressed in colorectal CAFs and correlate with poor patient survival and potential relapse [48].

The Involvement of CSCs in CRC Therapeutic Resistance

EMT Driven Stemness and Drug Resistance

EMT is a process by which epithelial cells take on a mesenchymal phenotype, resulting in the loss of their cell-to-cell adhesion ability and cellular polarity [49]. Elevated EMT markers are associated with cancer invasion and metastatic disease [50]. EMT processes drive the transition from benign tumors to malignant ones. Activation of EMT processes promotes cell stemness, giving rise to CSCs, which are associated with metastatic disease, cancer recurrence, and therapeutic resistance [51]. CSCs resemble EMT phenotype cells in terms of stemness and ability to promote metastasis as observed by elevated levels of EMT markers such as vimentin, fibronectin, and lower levels of E-cadherin. The EMT phenotype has the potential to generate CSCs via the activation of the Ras/mitogen-activated protein kinase (MAPK) signaling pathway [52]. Upregulation of the Ras/MAPK pathway is associated with increased mitotic activity in cancer cells, driven by hyperactivation of cancer specific BubR1 mitotic protein activities [53]. Different Ras isoforms have specific abilities to induce stemness in CSCs [54]. Kirsten rat sarcoma viral

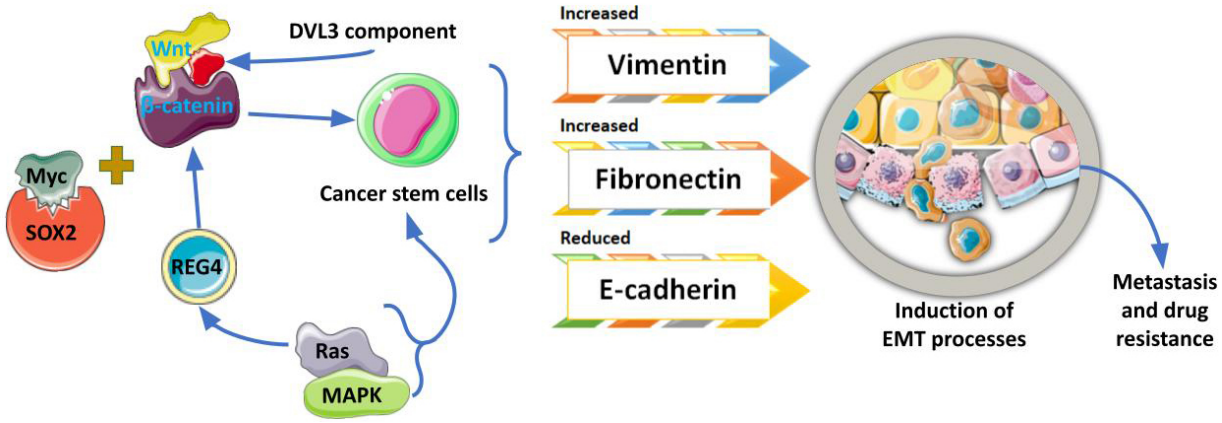

Fig. 3. Multiple pathways induce epithelial mesenchymal transition (EMT), resulting in therapeutic resistance associated with colorectal cancer (CRC) stem cells. About 40% of CRC cases carry Kirsten rat sarcoma viral oncogene homolog (*KRAS)* mutations. These mutations can independently induce the formation of cancer stem cells (CSCs) or act in conjunction with other oncogenes/transcription factors such as Wnt/β-catenin via *REG4*. The Myelocytomatosis (Myc)/sex determining region Y-box 2 (SOX2) signaling pathway collaborates with Wnt/β-catenin to induce a similar effect in the tumor microenvironment (TME). The dishevelled-3 (DVL3) protein, which is associated with Wnt/β-catenin, is highly expressed in the CRC TME and is involved in the regulation of CSCs and therapeutic resistance. All these factors induce the increased expression of EMT markers, vimentin and fibronectin, and downregulate the expression of E-cadherin. This leads to the initiation of EMT and ultimately metastasis and therapeutic resistance.

oncogene homolog (*KRAS)* mutations are found in approximately 40% of CRC cases [55]. *KRAS* mutations have been shown to induce CRC stem cells through *REG4*, which activates the Wnt/β-catenin pathway [56]. The dishevelled-3 (DVL3) protein is associated with the Wnt/β-catenin pathway and plays a role in cancer progression, therapeutic resistance, and promotion of stemness in cancers. In CRC samples, DVL3 is expressed at higher levels than in normal tissue samples. Inhibition of DVL3 has been shown to reverse methotrexate therapeutic resistance in CSCs by regulating the Notch signaling pathway [57]. Similarly, inhibiting DVL3 prevents CSC stemness, EMT, and therapeutic resistance to vincristine and oxaliplatin in CRC cancer xenografts. Furthermore, the stemness of CRC stem cells and EMT are facilitated by the Wnt/β-catenin pathway in conjunction with the Myc/SOX2 pathway [58] (Fig. 3).

The TME uses multiple mechanisms, including angiogenesis, to promote CSCs and cancer invasion. In the TME, cancer cells proliferate rapidly resulting in increased oxygen and nutrient uptake. Subsequently, hypoxic conditions activate angiogenic factor, hypoxia-inducible factor (HIF), which in turn activates pro-angiogenesis molecules such as vascular endothelial growth factor (VEGF) needed for neovascularization [59]. Hypoxia together with EMT are required for the stemness of cancer cells and metastasis. Angiogenesis factors use vasculogenic mimicry (VM) to stimulate neovascularization in cancer cells. The expression of hypoxia-inducible factor-1α (HIF-1α) upregulates VM. In this process, hypoxia induces the upregulation of EMT factors such as fibronectin1 (*FN1*) and vimentin. Using a 3-D culture system composed of rat tail collagen, the formation of vascular channels via VM correlated with invasiveness, migration, and stemness of HCT-116 and HT-29 CRC cell lines. Inhibiting EMT markers; E-cadherin, claudin-4, and vimentin, led to reduced VM in CRC cells [60]. Notably, CSCs can promote VM by differentiating into vascular-tube structures that pack together to form the vasculature in the absence of endothelial cells available for neovascularization [61].

TME Driven Metastasis and Therapeutic Resistance

The TME is responsible for the architectural structure that contributes to therapeutic resistance [62] (Fig. 4). The density of the TME depends on its acellular components including the extracellular matrix (ECM) and the vasculature. Cellular components include immune and stromal cells, while secretory proteins include cytokines, growth, and angiogenetic factors [63]. As tumors develop, they generate abnormal structural tension forces that influence tumor biology and progression. The TME mechanical infrastructure influences the diversity and heterogeneity of cancer cells [64] (Fig. 4). Common CRC markers, the Ras family of mutations contribute to the loss of structural integrity of the normal physiological tissue. This is achieved by altering actomyosin contractility and loss of epithelial base membrane organization, which facilitates invasion. The loss of cytoskeletal polarity is modulated by *KRAS* inhibition of phosphatases responsible for maintaining polarity [65]. Loss of cell polarity is mediated by EMT and is associated with metastasis [66].

Specifically, loss of the polarity protein, Pals1, in a xenograft of mouse CRC cells has been to enhance invasiveness and metastasis [67]. CAFs, which are also de-

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rived from MSCs [32], are an integral part of the TME architecture [68]. *KRAS* mutations can independently modulate the proliferation of cancer cells but require normal fibroblast-derived hepatocyte growth factor (HGF) to promote CRC cell invasion via modulation of C-mesenchymal epithelial transition (*MET*) expression [69]. Ding *et al*. [70] showed that CAFs-derived HGF fosters VM and angiogenesis, subsequently leading to metastasis through upregulation of phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) and extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) signaling. It is well established that the *KRAS* [71], VM [72], and EMT [73] mechanisms are associated with therapeutic resistance in CRC.

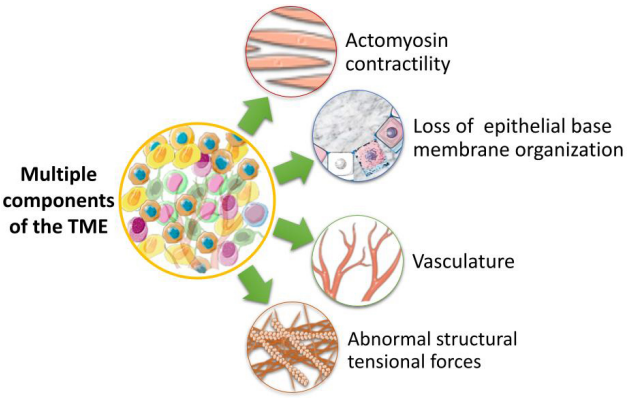

Fig. 4. Features of the colorectal cancer (CRC) tumor microenvironment (TME) contributing to metastasis and drug resistance. The TME is essentially responsible for the density and abnormal structural tensional forces that prevent drugs from penetrating the tumor, leading to drug resistance. Tumors have adapted to the harsh architectural structure by facilitating neovascularization to deliver oxygen and nutrients needed by their rapidly growing cells. The presence of CSCs induces epithelial mesenchymal transition (EMT) disrupting epithelial basement membrane organization, leading to cancer invasion and metastasis. EMT further contributes substantially to drug resistance. Moreover, the CRC Ras family of mutations contribute to cancer invasion, metastasis, and drug resistance by altering actomyosin contractility and inducing EMT.

Genomic Instability in CSCs Contribute to Therapeutic Resistance

Genomic instability is common in CRC and is actively being investigated for therapeutic potential [74]. In CRC, genomic instability typically manifests as chromosomal instability (CIN) mutations in distal tumors and microsatellite instability (MSI) in proximal–distal axis tumors. MSI tumors arise when DNA mismatch repair mechanisms are lacking and display a higher likelihood of *KRAS* mutations [75]. CIN mutations are observed in about 85% of sporadic CRCs [76]. Khot *et al*. [77] noted that the upregulation of *Twist1* induces CIN in CRC cells, accompanied by sub-chromosomal deletions and more double strand DNA breaks. *Twist1* plays a pivotal role in generating CSCs in the TME [78] and is known for its ability to induce EMT and therapeutic resistance [79]. *Twist1* regulates the expression of CSC markers such as *CD44*, sal-like protein 4 (SALL4), *NANOG*, myeloid ecotropic viral integration site 1 (*MEIS1*), growth differentiation factor-3 (GDF3), and sex determining region Y-box 2 (*SOX2*) thereby fostering the differentiation of CSCs [80] (Fig. 5).

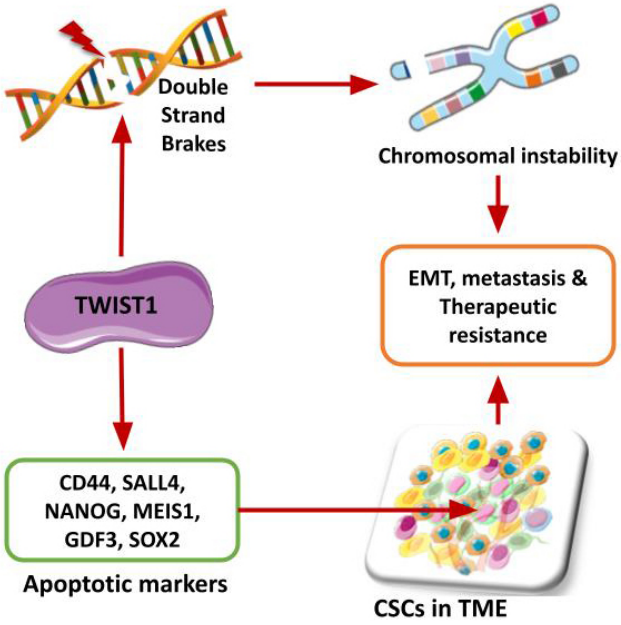

Fig. 5. *Twist1* plays a central role in cancer stem cells (CSCs) in the tumor microenvironment (TME) and induce therapeutic resistance. Twist related protein 1 (*Twist1*) initiates EMT, leading to the development of CSCs in the TME, promoting cancer metastasis and drug resistance. *Twist1* also induces chromosomal instability with double DNA strand breaks in CRC cells. Therapy resistant CSCs have been shown to induce genomic instability mutations, allowing them to survive and thrive. Moreover, *Twist1* leads to therapeutic resistance by modulating EMT markers, resulting in elevated levels of CSCs in the TME.

In tyrosine kinase inhibitor-treated leukemia stem cells, oxidative DNA damage resulted in genomic instability in Chronic Myelogenous Leukemia in Chronic Phase (CML-CP) like mice. The ensuing mutations were similar to those observed in tyrosine kinase inhibitor-resistant *BCR-ABL1* mutations, suggesting that genomic instability may contribute to therapeutic resistance [81]. Lagasse [82] describes genetic instability in CSCs as a “vehicle with the best engine” for cancer, especially since CSCs are able to accumulate stochastic mutations, promote spontaneous growth, and cause therapeutic resistance.

Table 1. Clinical trials evaluating the effectiveness of cancer stem cell therapy in colorectal cancer.

Clinical study	Purpose of the study	Trial number
A phase I/II study of active immunotherapy with cancer stem cells vaccine for colorectal cancer (CRC)	The study assessed anti-cancer immune responses in cytotoxic T-cells primed with colorectal cancer stem dendritic cells. Response of cancer stem B-cell antibodies from peripheral blood mononuclear cells stimulated with colorectal cancer stem dendritic cells.	NCT02176746
Invasiveness and chemoresistance of cancer stem cells in colon cancer	Characteristics of dissemination and chemoresistance of colorectal cancer stem cells and their genetic landscape.	NCT01577511
Changes in stem cells of the colon in response to increased risk of colorectal cancer	Frequency and distribution of stem cells in patients who are at high and normal risk of colorectal cancer. Increased cell proliferation at the top of the crypt in high risk patients are due to a change in the number of stem cells in the crypt base.	NCT01075893
CD133+ cell infusion in patients with colorectal liver metastases	Patients not eligible for surgery were treated with CD133+ and portal embolization to improve their eligibility for surgical intervention.	NCT03803241
Radiolabeled monoclonal antibody therapy plus peripheral stem cell transplantation in treating patients with metastatic or recurrent colorectal cancer or pancreatic cancer	Efficacy of combination therapy with radiolabeled monoclonal antibody and peripheral stem cell transplantation in metastatic or recurrent colorectal cancer that are unresponsive to previous treatment.	NCT00004087
Role of CD133 and microsatellite status in evaluation of rectosigmoid cancer young adults received neoadjuvant treatment	Correlation between microsatellite status and colorectal cancer stem cells and their association with disease outcome and therapeutic response.	NCT03002727
Feasibility study on stem cells sensitivity assay	Identify and isolate cancer stem cells in solid cancers including colorectal cancer.	NCT01483001
Cancer stem cell markers and prognostic markers in circulating tumor cells	Compare the genetic landscape of circulating and primary tumors to identify the frequency of genomic cancer cell profiles in a subgroup of patients with increased levels of circulating tumor cells or patients with early relapse.	NCT01286883

Table 2. Potential targets to increase sensitivity of cancer stem cells (CSCs) to oncotherapies.

Target	Action	References
miR-139-5p	Targets the *BCL-2* pathway thus inhibiting metastasis and increases sensitivity to chemotherapy.	[95]
Mitochondrial organelles	Morphological change resulting functional disability and reduced energy production needed by CSCs to de-differentiate.	[98]
GLUT1 metabolic gene	Inhibition of *GLUT1* by WZB117 resulting in reduced glucose uptake by CSCs.	[99]
KRAS–JNK axis	Attenuates stemness and self-renewal ability	[100]
miR-34a	Regulates stemness by targeting *NOTCH*, *MYC*, *BCL-2*, and CD44 stem cell markers.	[104]
miR-196a-5p	Inhibition in CD44+ cells prevented cancer invasion and EMT. MicroRNA promotes stemness by targeting *Smad4*.	[105]
miR-590-5p	Downregulates the expression of stemness marker, *SOX2*.	[106]
miR-200 family/miR-34	EMT by TGFβ.	[107–109]
miR-371-373	Targets Wnt/b-catenin.	[110]
Long coding RNA	Regulation of stemness by modulating the expression if stemness markers including *SOX2*, *OCT4*, and *NANOG*. Upregulation of a p53-responsive gene, *ITIH5* by long coding RNA LINC00261 reduced stemness properties and drug resistance.	[111,112]

In 2020, Safa [83] profiled studies demonstrating the role of CSCs in the TME in chemotherapeutic resistance. In CRC, CSCs have been implicated in therapeutic resistance via the involvement of CAF exosomes, upregulation of anti-apoptotic proteins, and regulation of the expression of microRNAs [83]. In 2018, Manic *et al.* [84] isolated CSCs from primary CRC tissues. They then screened multiple drugs to identify effective anti-CSC agents. Checkpoint kinase (CHK1) emerged as the most potent anti-CSC agent, which was achieved by inhibiting the DNA damage response in four out of the five CRCs investigated in the study [84]. The study revealed an abundance of *TP53* mutations, rendering CSCs sensitive to LY2606368. Sensitivity was specifically determined by stress response and

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H2A histone family member X (γH2AX) DNA damage response mutations. LY2606368 significantly induced DNA replication in responsive CRC stem cells. This effect was observed when CHK1 inhibition led to apoptosis, suggesting that CHK1 serves as a specific target for LY2606368. Nonresponsive CSCs expressed high levels of the p53 target cyclin-dependent kinase inhibitor 1A (CDKN1A/p21), suggesting that *TP53* mutations may serve as a biomarker for LY2606368 sensitivity [84].

CSCs Sensitization to Oncotherapies

Reprogramming the TME to sensitize CRC stem cells to immunotherapy is pivotal for overcoming treatment resistance. TME reprogramming can be achieved by normalizing the tumor vasculature by inhibiting the factors involved in angiogenesis and modulating the profile of immunosuppressive cells. Such efforts may be further enhanced by the combination of therapeutic agents [85]. Nonetheless, these approaches may prove sub-optimal if CSCs are not adequately considered. Given their heterogeneous nature, therapies that target CSCs are warranted to combat resistance, prompting several clinical trials (Table 1). Minimal residual disease, as well as concentrations of CSCs, is primarily responsible for cancer progression and metastasis. Most oncotherapies target rapidly proliferating cancer cells during the M or S phase of the cell cycle. However, CSCs, reside at the G0 phase of the cell cycle, rendering cell cycle-targeting drugs ineffective as anti-CSC agents. To overcome this, the F-box and WD40 repeat domain-containing protein 7 (Fbw7) regulates the cell cycle via the ubiquitin degradation of cyclin E and c-Myc, consequently activating cyclin-dependent kinase 2 (CDK2) and shifting the cell cycle from the G1 to the S phase [86]. Dysregulation of the G1-to-S phase switching mechanism is responsible for uncontrolled proliferation of cancer cells [87]. Fbw7 is highly expressed in therapy resistant CSCs, resulting in ubiquitin degradation of c-Myc and ultimate cell cycle arrest. Thus, the upregulation of Fbw7 induces cell cycle arrest, rendering CSCs dormant. Consequently, Fbw7 may be a potential therapeutic target for either abrogating CSCs or preventing cancer progression [88].

Synthetic compounds such as retinoid WYC-209 can also be used to inhibit the proliferation of CSCs and induce apoptosis. Retinoid WYC-209 has demonstrated greater efficacy in triggering apoptosis of CSCs than other anticancer drugs, including cisplatin, tazarotene, and all-trans-retinoic acid. CSCs placed on Arg–Gly–Asp-coated elastic round microgels and treated with retinoid WYC-209 for one hour showed substantially reduced traction forces and apoptosis 6 hours after treatment. F-actin was notably reduced [89], affecting the vascular integrity of the TME as well as metastatic potential [65].

In human CRC stem cells, CD133, a marker of CSCs, is highly expressed in adriamycin (ADR)-resistant cells. High levels of CD133 are positively correlated with the Multidrug resistance (MDR) protein 1/P-gp (*MDR1*) gene, which is associated with the doxorubicin-resistance signaling pathway. Overexpression of CD133 was demonstrated to be regulated by the activation of NF-κB by AKT, suggesting that blocking the PI3K/AKT/NF-κB signal transduction pathway could inhibit the expression of *MDR1*. Inhibition of CD133 could thus reverse CRC drug resistance by blocking the AKT/NF-κB/MDR1 pathway [90].

Lamichhane *et al.* [91] studied therapeutic resistance of CSCs to the MAPK pathway in *BRAF* and *KRAS* mutant CRC cells. MAPK inhibition led to the expression of the CSC markers, CD166 and aldehyde dehydrogenase 1A3. The sensitivity of CSCs to multiple drugs was then tested, and a combination of trametinib and mithramycin was found to be most potent in suppressing CSCs in CRC [91]. Additionally, the Notch signaling pathway contributes to maintaining stemness of CSCs in several cancers including CRC. The activity of the Notch signaling pathway is upregulated by about 10 to three times in CRC stem cells and promotes stemness by inducing EMT. Inhibiting the Notch signaling pathway re-sensitizes cancer cells to chemotherapy and inhibits stemness [92].

Several microRNAs (miRNAs) play a role in preventing stemness in CRC cells and improving therapeutic response [93]. These include miR-145, which inhibits the EMT transcription factor snail family transcriptional repressor 1 (*SNAI1*), and suppresses stemness in CRC cells. Repression of miR-145 also enhances the sensitivity of CRC cells to radiotherapy [94]. Conversely, downregulation of miR-139-5p promotes EMT-induced metastasis in CRC cells. Ectopic expression of miR-139-5p inhibits CRC cell invasion and metastasis while enhancing sensitivity to chemotherapy. miR-139-5p targets the BCL2 pathway to overcome therapeutic resistance, further attenuating CRC progression and metastasis [95].

Overexpression of miR-4666-3p inhibits stemness through TGF-βR1, whereas its downregulation targets interferon (IFN)-γR1/2 to prevent apoptosis in quiescent colon cancer stem cells. Low levels of miR-329 inhibit TGF-β1 secretion. Thus, miR-4666-3p and miR-329 synergistically block CRC initiation and stemness via the TGF-β/Smad pathway, which is crucial for maintaining stemness in colon CSCs [96].

Targeted Metabolic Reprogramming to Avert Therapy Resistance

Recent studies aim to understand the crosstalk between CSCs and the TME to develop novel therapies capable of curtailing the stemness that is maintained by the TME. These therapies aim to impede metastasis and therapeutic resistance, but tumor heterogeneity driven by CSCs remains a major challenge. The availability of vast molecular data presents the conundrum of identifying precise

molecular markers amenable to targeting in the pursuit of novel oncotherapies. Consequently, targeting specific pathways, such as metabolic and immune signals involved in CSC transformation, may prove advantageous [97].

Reprogramming of metabolic signatures in radiotherapy resistant CSCs depends on glycolysis in the demanding anaerobic conditions of the TME. This provides for the elevated energy requirements needed for differentiation and rapid growth of CSCs. Notably, CSCs display distinctive mitochondrial morphology characterized by altered distribution and shape when compared to that of parental cells. While mitochondria organelles typically scatter throughout cytoplasm; in CSCs, mitochondria exhibit peri-nuclear localization. Moreover, CSCs also exhibit more small globules and linear tubules with fewer branched tubules. These distinctive features may serve as indicators of suitability for targeted therapy [98] (Table 2, Ref. [95,98–100,104–112]).

Low or normal glucose levels reduce CSC stemness as indicated by the expression of glial fibrillary acidic protein. WZB117 inhibits the metabolic gene, *GLUT1*, which is required for the maintenance the TME. WZB117 reduces glucose uptake and the expression of CSC markers. Shibuya *et al*. [99] injected WZB117-treated cells into nude mice and noted no tumor growth compared to control treated cells. Similar outcomes were observed when WZB117 was systemically administered in xenografts with untreated cell lines [99]. Consequently, *GLUT1* emerges as a potential therapeutic target for CSCs [99]. The *KRAS–JNK* axis has also been identified as a target against CSC stemness and cancer initiating ability [100]. Apart from *KRAS*, several other CRC related mutations such as *APC*, *TP53*, and *SMAD4* have been shown to be involved in metabolic reprogramming by regulating metabolic enzymes in CSCs [101]. Drugs that target mitochondria have also shown potential. CRC stem cells manipulate mitochondria to increase the expression of anti-apoptotic proteins, promote mitophagy responsible for the production of reactive oxygen species (ROS), and addiction to oxidative phosphorylation (OXPHOS), promoting therapeutic resistance [102]. OXPHOS inhibitors could be combined with immunotherapy to induce the ability of the immune system to fight off cancer and prevent drug resistance [103].

Conclusions

CRC stem cells are key factors in immunotherapy and personalized medicine. These unique sub-populations of cells exhibit high tumor heterogeneity and plasticity and self-renewal abilities. Unlike other types of cancer cells, they reside in the G0 phase of the cell cycle phase, which makes them difficult to treat using conventional therapies. CSCs are key components of the TME, promoting cancer initiation, development, advancement, relapse, and therapeutic resistance. The synergistic interaction between CSCs and the TME warrants urgent elucidation of these interacting mechanisms to effectively overcome therapy resistance in CRC. This may include using combination therapies that target the synergistic effects of this mutual relationship. Understanding CRC stem cell biology and the TME is pivotal for averting therapy resistance and understanding the mechanisms of metastasis.

Author Contributions

BPD, RM, TVM and ZD conceptualized the review. BPD prepared and wrote the original draft. BPD, RM, TVM and ZD contributed to writing, editing and reviewing the paper. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Rahaba Marima, Botle Precious Damane and Zodwa Dlamini are serving as one of the Guest editors of this journal. We declare that Rahaba Marima, Botle Precious Damane and Zodwa Dlamini had no involvement in the peer review of this article and has no access to information regarding its peer review.

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