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REVIEW ARTICLE

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# Role of epithelial-to-mesenchymal transition and cancer stem cells in colorectal cancer

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## ABSTRACT

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths. To properly investigate the biology of the tumor and the molecular mechanisms leading to cancer progression or treatment resistance, it seems imperative to explore the key pathways like epithelial-to-mesenchymal transition (EMT) and cancer stem cells (CSCs). This review aimed to collect up-to-date knowledge on the subject of EMT and CSC in colorectal malignancies. In CRC, both EMT and CSC are associated with aggressive tumor behavior, metastases, cancer recurrence, and chemotherapy resistance. Due to their close relationship, the potential for targeting these pathways as therapeutic interventions is promising. However, direct usage of EMT and CSCs as therapeutic targets requires further investigation. Future studies should focus on unraveling the complex mechanisms underlying EMT and CSC involvement in CRC progression and developing tailored therapeutic strategies with acceptable toxicity profiles and minimal adverse events.

**Keywords**: colorectal cancer, epithelial-to-mesenchymal transition, cancer stem cells

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## Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer-related deaths, accounting for approximately 10% of all annually diagnosed cancers and cancer-related deaths worldwide [1, 2]. In highly developed countries, stabilizing or decreasing trends are observed, primarily attributed to nationwide screening programs detecting precancerous lesions. Unfortunately, in recent years the overall incidence of CRC has increased in individuals younger than 50 years of age, especially rectal and left-sided colon cancer [3, 4]. Both hereditary and environmental risk factors take part in the development of CRC. Positive family history appears to be related to approximately 10–20% of all cases of CRC [3]. Other established risk factors encompass increasing age, male sex, obesity, red meat intake, and alcohol consumption [5, 6]. According to the National Institute for Health and Professional Excellence, suspected CRC recognition and referral for further diagnostic process should be related to rectal bleeding, abdominal mass, change in bowel habits, unexplained weight loss, abdominal pain, and iron-deficiency anemia [7].

Most cases of CRC arise from precursor adenomatous or serrated polyps, which creates the opportunity for prevention through the detection and removal of precancerous lesions before they can progress to overt malignancy [5]. This multistep progression involves a series of histological, morphological, and genetic changes accumulating over time [8]. The accumulation of acquired genetic and epigenetic changes leads to

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**Table 1. Markers and transcription factors of epithelial-mesenchymal transition**

| Epithelial cell markers (downregulated) | Mesenchymal cell markers (upregulated) | Transcription factors |
|---|---|---|
| E-cadherin | N-cadherin | SNAIL |
| Claudins | Vimentin | SLUG |
| Occludin | Fibronectin | TWIST |
| MUC-1 | Alpha-SMA | ZEB1 |
| Desmoglein-3 | MMP2 | |
| Desmocollin | MMP9 | |
| | Collagen I | |

alpha-SMA — alpha Smooth Muscle Actin; E-cadherin — epithelial cadherin; MMP — matrix metalloproteinase; MUC-1 — mucin-1; N-cadherin — neural cadherin; SLUG — snail family transcriptional repressor 2; SNAIL — snail family transcriptional repressor 1; ZEB1 — zinc-finger E-box-binding 1

the transformation of normal glandular epithelial cells and ultimately results in the development of invasive adenocarcinomas. Numerous molecular alterations playing a role in the initiation and progression of colon polyps have been identified over the last three decades. [9]. The cell of the origin for the majority of CRC cases is assumed to be a cancer stem cell (CSC) or stem-cell-like cell [10]. To properly investigate the biology of the tumor and molecular mechanisms leading to cancer progression, it seems imperative to explore the key pathways like epithelial-to-mesenchymal transition (EMT) and CSC as they are reported to promote more aggressive behavior [11]. This review aimed to collect up-to-date knowledge on the subject of EMT and CSC in colorectal malignancies.

## Epithelial-to-mesenchymal transition

The epithelial-to-mesenchymal transition (EMT) is a reversible cell-biological program that converts epithelial cells into more mesenchymal cell states. However, recent studies consider EMT during tumor progression as a set of multiple dynamic states between epithelial and mesenchymal phenotypes, rather than full phenotypic transformation [12–14]. Cancer cells undergoing EMT demonstrate morphological changes along with molecular alterations [15]. Epithelial cells are characterized by epithelial cell-to-cell junctions and apical-basal polarity, while mesenchymal ones present spindle-like morphology and enhanced motility [14]. Depending on the signaling contexts, epithelial cells may lose only some characteristics or show both epithelial and mesenchymal properties [16]. The hallmark of EMT is based on the loss of epithelial marker expression, especially Epithelial cadherin (E-cadherin) with simultaneous acquiring higher expression of mesenchymal markers, such as N-cadherin and vimentin [15, 17]. The repression of epithelial phenotype and activation of the mesenchymal one is orchestrated by the changes in gene expression, directly and indirectly, driven by numerous transcription factors, including TWIST, snail family transcriptional repressor 1 (SNAIL), and zinc-finger E-box-binding (ZEB) [16]. Examples of markers and transcription factors associated with EMT are presented in Table 1.

## Epithelial-to-mesenchymal transition in colorectal cancer

Epithelial-to-mesenchymal transition in colorectal cancer correlates with cancer cell invasiveness and metastasis, facilitating cell mobility and dissemination [18, 19]. The overexpression of EMT transcription factors plays a key role in increasing the risk of distance metastasis as well as decreasing overall survival in CRC patients [20]. Up-regulation of snail family transcriptional repressor 2 (SLUG) and vimentin expression was proposed as potential predictive biomarkers for identifying patients with lymph node metastasis and worse prognosis in CRC [21, 22]. Interestingly, SLUG suppression induced a high reduction of vimentin expression in CRC cell lines and resulted in decreased rates of cell proliferation, invasion, and migration. In conclusion, overexpression of SLUG with simultaneously increased vimentin expression facilitates mesenchymal phenotype and helps promote CRC cell invasiveness and metastasis [21].

Tumor cells exhibiting features of EMT are often observed histologically as tumor budding – isolated single cancer cells or clusters of up to four cancer cells located at the invasive front of the tumor. However, there is a possibility that both EMT and tumor budding are hallmarks of a more aggressive tumor phenotype rather than EMT directly causing tumor budding [23]. Budding in CRC is an important morphological reflection of the invasive activity that precedes metastasis and has been shown to be an independent predictor of poor outcomes [24]. The EMT process in tumor budding cells is indicated by mesenchymal morphology with ZEB1 and SNAIL positivity with concomitant loss of E-cadherin expression [25].

Ample evidence suggests that tumors undergoing EMT might resist conventional drug therapies [26]. For example, oxaliplatin-resistant CRC cells present mesenchymal morphology (loss of polarity, spindle shape, increased mobility) with EMT-suggestive features such as decreased E-cadherin and increased vimentin expression [27]. Zinc-finger E-box-binding 1 knockdown was shown to effectively restore oxaliplatin-sensitivity in CRC cells by reversing EMT [28]. Moreover, EMT was reported to be implicated in the microRNA-driven modulation of tumor cells' response to 5-fluorouracil and oxaliplatin [29]. On the other hand, oxaliplatin is suspected to induce EMT by promoting the release of reactive oxygen species (ROS). The pre-treatment with ROS scavenger — N-acetyl-L-cysteine was shown to inhibit oxaliplatin-induced EMT and progression [30]. Nevertheless, it remains unknown how exactly EMT influences drug resistance. Some studies suggest that EMT might generate cells with CSC properties, resistant to conventional anticancer regimens [31].

Epithelial-to-mesenchymal transition-related markers can potentially serve as biomarkers to guide personalized treatment decisions in CRC [21, 22]. Moreover, evaluating the EMT status of individual tumors may potentially facilitate the prediction of response to specific therapies, such as targeted agents or immunotherapies. Emerging evidence suggests that tailoring treatment based on the EMT characteristics of the tumor could lead to more effective and personalized treatment strategies. Recent immunotherapeutic methods have achieved remission in a substantial portion of cancer patients who were previously deemed untreatable. For instance, EMT induced by TGF-β leads to the exclusion of T cells, but inhibiting TGF-β using antibodies or small molecules has shown potential in enhancing the tumor's response to programmed death-ligand 1 (PD-L1) blockade in preclinical studies [32, 33]. While preclinical evidence suggests a rationale for combining TGF-β inhibitors, such as galunisertib or TGF-β blocking antibodies, with anti-PD1 therapy, it is important to note that the true clinical significance of this combination is yet to be determined. Although the concept of reversing immune suppression induced by the EMT with specific drug combinations is noteworthy, robust clinical evidence supporting its efficacy is lacking. Further clinical trials are warranted to elucidate the therapeutic potential of combining TGF-β inhibitors with immune checkpoint inhibitors (ICI) in cancer treatment [32, 33].

Other new therapeutic targets include signaling pathways that regulate the proliferation and differentiation of stem cells. The Notch pathway is one of the major developmental signaling pathways and comprises four homologs (Notch1-Notch4) transmitting growth signals. Studies have associated Notch signaling with colorectal tumorigenesis, examining its expression in cancer tissue specimens [34]. Notch directly activates EMT by promoting nuclear translocation of the Notch intracellular domain (NICD), which stimulates the expression of EMT transcription factors like Snail. Notch interaction can extend to NICD cleavage by disintegrins and γ-secretase [17]. Tarextumab (OMP-59R5), a monoclonal antibody targeting Notch receptors, is undergoing phase I and II trials for various solid tumors, with or without combined chemotherapy. A γ-secretase inhibitor, RO4929097 (RG-4733), has been tested in phase II trials for metastatic colorectal cancer, but with limited effectiveness as a single agent [34]. MK-0752, another γ-secretase inhibitor, has shown clinical benefits and tolerability in phase I trials for advanced solid tumors [35].

## Epithelial-to-mesenchymal transition marker: E-cadherin

Epithelial cadherin is a large single-pass transmembrane glycoprotein involved in calcium-dependent cell-cell adhesion molecules [36]. It is the core component of epithelial adherens junctions, pivotal for tissue development, differentiation, and maintenance [37]. Epithelial cadherin has three domains — extracellular, transmembrane, and intracellular. The extracellular domain harbors five cadherin repeats allowing cadherin to form homotypical interaction in the presence of extracellular calcium. As a result of this interaction, a tight zip-like structure between two cells is formed to provide the strongest cell-cell adhesion mechanism in the epithelium [38]. The intracellular, highly conserved, cytoplasmic domain is used by E-cadherin to bind with certain catenin family proteins, mainly composed of b-catenin and p120-catenin. These different binding partners mediate and regulate the activity of E-cadherin. They yield a multi-protein complex interacting through a-catenin to actin filaments in the cell. A clustering of cadherin-catenin complexes on the cell membrane leads to local remodeling of intracellular actin and microtubules and facilitates the formation of cell-cell adherens junctions [39–41].

Downregulated E-cadherin expression in CRC indicates the presence of lymph node metastases and a worse prognosis [22, 42, 43]. Additionally, it is a significant factor influencing adverse clinicopathological features, such as low differentiation, vascular and lymphatic invasion, deep infiltration, higher TNM, and Duke's stages [44]. In the CRC specimen, it was demonstrated that the Snail2 transcription factor promotes EMT occurrence by downregulating the expression of E-cadherin through Snail2 interaction with histone deacetylase and the polycomb repressive complex 2 recruitment [45]. Moreover, E-cadherin is a binding point of

*Fusobacterium nucleatum* adhesin — FadA. FadA binds to E-cadherin, activates β-catenin signaling, and increases the inflammatory and oncogenic response [46]. E-cadherin also regulates the proliferation of colorectal cancer stem cells [47].

## Cancer stem cells

The role of undifferentiated cells in cancer — possibly CSCs — was first mentioned by Rudolf Virchow in 1855 [48]. Further studies hypothesized that tumor growth is driven by CSCs — a small population of cells with the characteristics of embryonic stem cells [49]. Cancer stem cells possess a capacity for unlimited self-renewal, which leads to their immortality [50, 51]. They undergo either a symmetrical, or asymmetrical, self-renewal process during cell division. Symmetrical cell division generates two identical daughter CSCs. In contrast, asymmetrical cell division produces one daughter CSC and one differentiated progenitor cell, which results in the expansion of the number of CSCs with the tumor growth [52]. Unlike embryonic stem cells, CSC loses control of replication and differentiation, which may lead to uncontrolled tumor genesis. They can differentiate into a large heterogeneous population of tumor cells with altered phenotypes [53]. Cancer stem cells express antigens at lower levels thus they are difficult to target. Their identification is based mainly on the presence of populations of cells that have stem cell-like properties [52]. Some authors distinguish stationary CSCs and migrating CSCs. Stationary CSCs are involved in each step of tumor progression while migrating CSCs, which have undergone EMT, form metastases [54]. Targeting CSCs has been the focus of research for many years and the combination of CSC-targeted therapies with conventional non-targeted therapies might result in decreased chemoresistance [55]. The exact mechanisms by which CSCs can escape chemotherapy treatment appear diverse [56]. Some potential mechanisms are presented in Figure 1 [52, 56, 57].

## Cancer stem cells in colorectal cancer

According to recent studies CRC pathogenesis might be induced by transformed CSCs with the ability to self-renew and to differentiate aberrantly [58]. Colorectal cancer stem cells (CR-CSCs) are heterogeneous cells and their classification is based on their molecular and functional features such as self-renewal, pluripotent, and plasticity capabilities [59]. Colorectal cancer tumor biology should be analyzed by the clonal expansion and repopulation characteristics of the various CSC cell lineages within the tumor. A recent study showed that human LGR5+ colorectal cancer cells can serve as CSCs in growing cancer tissues. Lineage-tracing experiments confirmed their self-renewal and differentiation capacity. Ablating LGR5+ CSCs leads to tumor regression, followed by regrowth driven by re-emerging CSCs. KRT20 knock-in reporter marks differentiated cancer cells that can revert to CSCs and contribute to regrowth. Combined chemotherapy enhances the targeting of LGR5+ CSCs, revealing their therapeutic potential in colorectal cancer [60]. Colorectal cancer based on CSCs might originate from an intestinal stem cell (single mutation), cells in transit, and terminally differentiated cells (at least one transforming and one de-differentiating mutation). Stemness is further responsible for 3 main processes triggering tumor progression — cell growth and proliferation, recurrence and metastases, and therapy resistance [61]. Colorectal cancer stem cells were confirmed to be closely related to CRC resistance to chemotherapy [62]. Colorectal cancer stem cells are not sensitive to 5-fluorouracil and oxaliplatin, which may be one of the key factors leading to metastasis and recurrence after standard chemotherapy [63]. Current studies and therapies focus on targeting surface markers or cellular signal pathways of CR-CSCs, promoting the differentiation of CSC, or aiming at the tumor microenvironment affecting CSC properties [64]. Nevertheless, since CR-CSCs share common characteristics with normal stem cells, the biggest challenge is to identify specific markers and techniques that exclusively target cancerous ones while sparing healthy normal stem cells [65].

## Cancer stem cell marker: ALDH1

Aldehyde dehydrogenase 1 (ALDH1) is a cytosolic enzyme responsible for oxidizing a wide variety of intracellular aldehydes to their corresponding carboxylic acids [66]. Aldehyde dehydrogenase 1 has three main isotypes, ALDH1A1, ALDH1A2, and ALDH1A3 [67]. The members of the ALDH family present high similarity in structure and function. The global structural similarities support identical residues at the active site for aldehyde oxidation [68, 69]. Aldehyde dehydrogenase 1 is considered a marker for normal stem cells as well as cancer stem cells in several tissue types [70]. It is one of the most frequently used biomarkers in CSC-related studies [71]. Aldehyde dehydrogenase 1 has a role in the early differentiation of stem cells through its function in the oxidation of retinol to retinoic acid [72]. Retinoid signaling pathways are implicated in normal and cancer stem cells. Retinoic acid with its derivatives is involved in various physiological and pathological processes, including the regulation of gene expression, morphogenesis, development, self-renewal, and tumor resistance [73]. Retinoic acid regulates gene expression

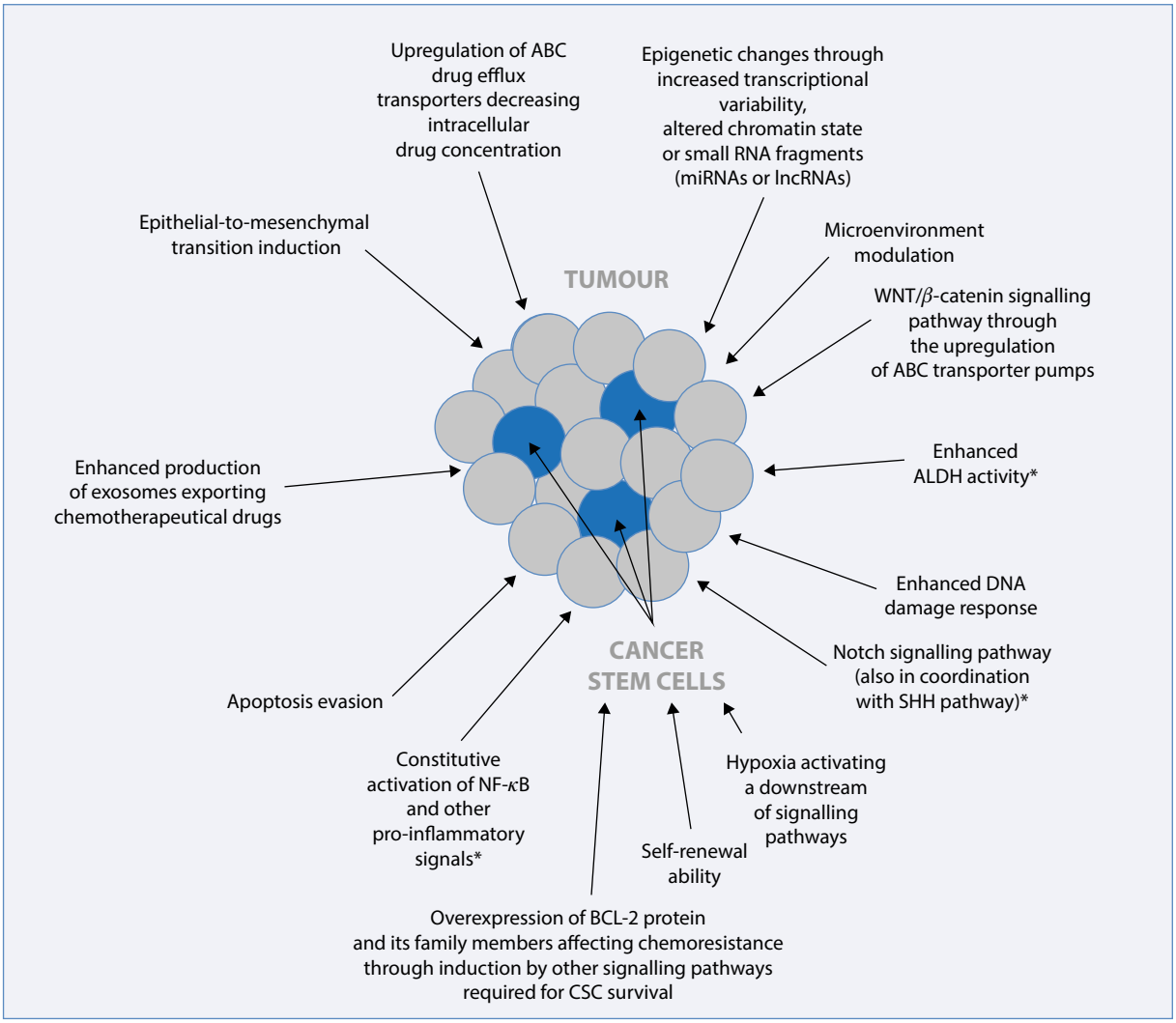


**Figure 1.** Scheme of potential mechanisms of cancer stem cell chemoresistance [52, 56, 57]; *direct mechanism not elear; ABC — ATP-binding cassette; ALDH — aldehyde dehydrogenase; BCL-2 — B-cell lymphoma-2; CSC — cancer stem cell; DNA — deoxyribonucleic acid; lncRNA — long non-coding RNA; miRNA — microRNA; NF-κB — nuclear factor kappa-light-chain-enhancer of activated B cells; RNA — ribonucleic acid; SHH — Sonic hedgehog

by serving as a ligand for nuclear retinoic acid receptors and retinoid X receptors [67].

High ALDH1 expression in CRC was shown to be an independent prognostic factor for poor prognosis and to correlate with higher tumor stage, nodal involvement, and grading [74]. Further studies found a highly statistically significant relationship between ALDH1 expression and lymphovascular invasion, degree of lymphocytic infiltration, budding, and the American Joint Committee on Cancer (AJCC) stage [75]. Moreover, two independent studies correlated high expression of ALDH1 with the early onset of CRC [11, 76]. Colorectal cancer patients with high ALDH1 expression detected before chemoradiation were shown to have a higher recurrence rate within 36 months postresection [77]. Aldehyde dehydrogenase 1 also seems to be a promising marker for identifying CR-CSC and a potential candidate for directed therapy due to the low expression of ALDH1 in the normal colon compared to the tumor specimen [67, 78].

## Epithelial-to-mesenchymal transition and cancer stem cells

Various research demonstrated that EMT markers and stem cell markers are co-expressed in circulating tumor cells derived from patients with metastasis. Moreover, EMT induction or activation of EMT transcription factors confers stem-like features in

cancer cells, across a wide variety of human carcinoma types [56]; nevertheless, the exact mechanistic link between EMT and CSC status remains elusive. Possibly, the EMT program induces alterations in the spectrum of proteins secreted by cancer cells, establishing autocrine signaling loops, which in turn are essential for the induction and maintenance of stem-cell properties [79]. The potential link between EMT and CSCs might also be a key to acquisition of resistance to cancer drugs and cancer cell plasticity, in which the cancer cells transform into malignant ones [80]. The expression of the EMT-associated gene provides cancer cells with invasive and metastatic characteristics, resistance to therapies, and CSCs phenotypes on cancer cells [61]. Epithelial-to-mesenchymal transition-program activation is essential for physical dissemination of cancer cells to distant organs, but also for entrance into the CSC state, which enables disseminated cells to serve as founders of metastatic colonies and, as a result, to colonize successfully foreign tissues [79]. Epithelial-to-mesenchymal transition, through interacting with the surrounding extracellular matrix proteins, was shown to enable in CSC the efficient development of mature adhesion plaques, which triggers signaling pathways to activate cell proliferation [81, 82]. Inhibitor agents with the primary purpose of inhibiting EMT were shown not only to inhibit EMT but also to suppress the stem cell-like properties [83]. The relationship between EMT and CSC is not one-way – some researchers even reported the influence of transcription factors associated with CSC in the regulation of EMT [84].

In terms of CRC, so far only individual studies have analyzed the interplay of CSC and EMT. The overexpression of both EMT and CSC markers was established in the CRC specimen [11]. The combination of altered CSC and EMT markers was associated with aggressive clinicopathological factors and shorter disease-free survival and overall survival [85]. Another study also demonstrated CSC and EMT markers as adverse prognostic factors and correlated their expression with *Fusobacterium nucleatum* level in tumor tissues, suggesting the potential involvement of this bacteria in EMT-CSC interplay [86]. Moreover, the expression of both CSC and EMT proteins was shown to be downregulated by silencing one long intergenic non-protein coding RNA 01315 (LINC01315) [87]. Forced expression of tumor-derived human growth hormone stimulated EMT and enhanced CSC-like behavior of CRC cells [88].

## Conclusions

The findings presented in this study underscore the significant association between EMT and CSCs with aggressive tumor behavior, metastases, and chemotherapy resistance in CRC. While targeting EMT and CSC properties holds promise as a novel therapeutic approach, the complex interplay between these processes necessitates further investigation. Future research endeavors should concentrate on clarifying the mechanistic connections between EMT and CSCs, as well as developing personalized treatment strategies that disrupt these pathways based on individual tumor characteristics. However, translating these findings into clinical practice poses challenges due to limited data on specific therapeutic interventions targeting EMT and CSCs. Collaborative multidisciplinary research initiatives are essential to overcome these challenges and accelerate the translation of promising therapeutic strategies into clinical applications, ultimately improving treatment response and patient outcomes in CRC.

## Article Information and Declarations

### Author contributions

M.F.: conceptualization, data analysis, writing — original draft; A.M.: writing — original draft; A.B.K.: conceptualization, supervision, validation, writing — original draft; writing — review and editing; A.D.: supervision, validation, writing — original draft, writing — review and editing.

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### Conflict of interest

The authors declare no conflict of interest.

### Supplementary material

None.

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