Concise Review: Aggressive Colorectal Cancer: Role of Epithelial Cell Adhesion Molecule in Cancer Stem Cells and Epithelial-to-Mesenchymal Transition

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Key Words. Colorectal cancer • Epithelial cell adhesion molecule • Cancer stem cells • Epithelial-to-mesenchymal transition • Cancer treatment • Therapeutic antibody

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ABSTRACT

Colorectal cancer (CRC) is one of the most common malignancies worldwide. In spite of various attempts to ameliorate outcome by escalating treatment, significant improvement is lacking particularly in the adjuvant setting. It has been proposed that cancer stem cells (CSCs) and the epithelial-to-mesenchymal transition (EMT) are at least partially responsible for therapy resistance in CRC. The epithelial cell adhesion molecule (EpCAM) was one of the first CSC antigens to be described. Furthermore, an EpCAM-specific antibody (edrecolomab) has the merit of having launched the era of monoclonal antibody treatment in oncology in the 1990s. However, despite great initial enthusiasm, monoclonal antibody treatment has not proven successful in the adjuvant treatment of CRC patients. In the meantime, new insights into the function of EpCAM in CRC have emerged and new drugs targeting various epitopes have been developed. In this review article, we provide an update on the role of EpCAM in CSCs and EMT, and emphasize the potential predictive selection criteria for novel treatment strategies and refined clinical trial design. STEM CELLS TRANSLATIONAL MEDICINE 2018;7:495–501

SIGNIFICANCE STATEMENT

Although the epithelial cell adhesion molecule (EpCAM) is a prominent tumor antigen of carcinomas, its therapeutic potential is far from being fully exploited. One reason for this might be its structural complexity and its paradoxical counter-regulation in discrete, but functionally converging tumor cell states, such as stemness and epithelial-to-mesenchymal transition. This study critically discusses the role of EpCAM variants in aggressive colorectal cancer (CRC). The main conclusion is that EpCAM depicts a promising CRC target, but that future drug development efforts should focus more on compounds that target the intracellular domain of EpCAM, rather than surface epitopes.

FUNCTION OF EpCAM

The epithelial cell adhesion molecule (EpCAM or CD326) is a transmembranous glycoprotein expressed on the surface of healthy epithelial cells. It consists of an extracellular (EpCAM extracellular domain (EpEX)), a short intramembranous, and a single intracellular domain (EpCAM intracellular domain (EpICD)). In malignant tumor cells, Maetzel et al. were able to show that shedding of EpICD to the nucleus leads to activation of the β-catenin/c-Myc pathway resulting in tumor cell proliferation [1]. Thus, overexpression of EpCAM is associated with cancer progression and poor outcome in several tumor entities including gastric [2] and pancreatic cancer [3]. EpCAM is a poor prognosticator particularly in colorectal cancer (CRC), where loss of membranous EpICD associates with unfavorable outcome as compared to patients with predominant expression of the full-length form [4–6].

In normal human tissues, expression of EpCAM is restricted to the basolateral membrane of epithelial cells within adhesions mediated by members of the cadherin family of proteins. The EpCAM molecule accumulates in cadherin-independent clusters but is absent from tight junctions and desmosomes [7]. In early studies by Balzar et al., EpCAM was proposed to be a cell-to-cell adhesion molecule able to increase cellular adhesion on extracellular matrix and other substrates [8]. However, other groups, using independent model systems, were able to only partially validate this finding. Enhanced adhesion might therefore depict a complementary—rather than main—function of EpCAM in association with other surface proteins and can mostly be expected in cells with a poorly shaped or defective adhesion machinery. Winter et al. showed that EpCAM modulates cadherin-mediated contacts by antagonizing E-cadherin (CD324), hence shifting adhesions from strong to weak [9]. Along similar

lines, EpCAM can also interact with the actin cytoskeleton. It is tempting to speculate that the proteins might compete for cognate binding sites on actin [10]. EpICD can interact with α-actinin. Intriguingly, the accumulation of α-actinin in EpCAM-mediated adhesions occurs independently of the accumulation of talin, vinculin, and α- and β-catenins, which are all pivotal components of focal adhesion complexes. α-actinin mediates binding to the cytoplasmic domain of several other molecules including β1-, β2-, β3-integrins, ICAM-1 (CD54), and L-selectin (CD62L) [8]. The negative modulation of cadherin-mediated cell adhesion by EpCAM is thought to involve a disruption of the interaction between α-catenin and filamentous actin [11]. Defective cell-to-cell contacts favor proliferation, migration, differentiation, and tissue maintenance, suggesting EpCAM as a prometastatic molecule [12]. Interestingly, transient downregulation of EpCAM is linked to epithelial-to-mesenchymal transition (EMT) in tumor cells, which is thought to favor cell motility and promote migration [12]. Furthermore, recent data showed that overexpression of EpCAM promotes EMT in tumor cells through increased levels of Slug, activation of the phosphatase and tensin homolog (PTEN)/protein kinase B (Akt)/mechanistic target of rapamycin (mTOR) signaling pathway, and downregulation of PTEN [13]. Overall, EpCAM appears to have distinct functions under different conditions, and context-dependent regulation is likely.

Interestingly, EpCAM is also centrally involved in development of the healthy colorectum. In fact, mutations or deletions in the EpCAM gene lead to congenital tufting enteropathy [14] and Lynch syndrome [15], respectively. Other studies revealed an even more versatile role of the EpCAM antigen. EpCAM is over- or de novo expressed on many types of normal and malignantly transformed epithelial progenitor cell populations and potentially plays a role in proliferation, cell movement, differentiation, and morphogenesis [16].

STEMNESS PROGRAMS AND EMT GOVERN CRC HETEROGENEITY AND PROGRESSION

CRC defines a heterogeneous tumor entity with several known molecular subtypes recently summarized as consensus molecular subtypes (CMS) 1–4 and CMS$_{mixed-feature}$ [17]. This novel classification takes into account various molecular and histological features such as microsatellite instability, immune activation status, morphological appearance (epithelial vs. mesenchymal), activation of Wnt, c-Myc and TGF-β signaling circuits, and metabolic dysregulation. In addition, prototypical CRC genes such as *TP53*, *APC*, *CTNNB1*, *SMAD4*, *PI3KCA*, *NRAS*, *PTEN*, *KRAS*, and *BRAF*, which are not strictly associated with the CMS classification system [17], further diversify the clinical presentation spectrum of CRC. Nevertheless, particular aberrations can provide a basis for personalized treatment decisions, such as activating mutations in *KRAS*, which serve as contraindications for epidermal growth factor receptor (EGFR)-targeted therapy [18].

A major obstacle for advancing precision medicine approaches for solid cancers is cellular variation within individual tumors commonly referred to as tumor heterogeneity. In CRC, single cell analysis of a plurality of protein antigens revealed extensive tumor heterogeneity [19] and multi-region sequencing in conjunction with copy number profiling showed site-specific genomic landscapes as well as branched evolution among primary and liver-metastatic lesions [20]. This can lead to lesion-specific treatment responses

[21] and resistance to EGFR blockade involving clonal evolution processes beyond clinical progression at the primary affected tissue (e.g., in blood) [22]. Apart from this type of heterogeneity that emerges over decades from genetic mutation, carcinomas also underlie swift regulation by epigenetic [23] and microenvironmental [24] means that concertedly act on distinct tumor cell subpopulations, such as cancer stem cells (CSCs), and furthermore trigger transitions between epithelial and mesenchymal states. The resulting plasticity generates a highly dynamic tumor ecosystem with extensive functional diversity among cancer cell subsets, which exacerbates malignancy and ultimately promotes drug resistance.

It is believed that CRC arises from the malignant transformation of an intestinal stem cell (ISC) [25] or, alternatively, from a more differentiated cell that has fate plasticity thus holding dedifferentiation potential [26]. Accordingly, molecular signatures specific for ISCs identify colorectal CSCs and further filter out patients at high risk for recurrence [27]. Several markers have been reported to define colorectal CSCs, but CD133 [28], Lgr5 [29], and CD44 [30] seem to be most reliable. Importantly, colorectal CSCs co-express EpCAM [30], are a source of drug resistance [31], and readily produce tumors upon transplantation into recipient mice [28]. They further have multilineage differentiation potential [32] and might be heterogeneous themselves [33, 34], hence directly contributing to cellular tumor complexity.

The EMT is another essential player in the heterogeneous make-up of carcinomas. This developmental process is frequently reactivated in CRC [17] and contributes to heterogeneous tumor cell phenotypes that cross lineage boundaries. Of note, EMT is associated with a more aggressive tumor cell behavior and furthermore confers resistance to anticancer drugs [35]. The concerted action of CSCs and EMT thus produces a degree of heterogeneity that escalates malignancy and poses serious therapeutic challenges.

CSCs [36] and EMT [37] are key drivers of metastatic CRC progression, and accordingly, their surrogate markers bear prognostic significance [17, 38]. At the invasive front, colorectal CSCs (or tumor cells that have adopted migratory potential through EMT) detach from the primary site and intravasate into nearby vessels. They survive in circulation and extravasate to seed metastases in classic target organs such as liver and lung. While during invasion, migratory properties and anoikis resistance are required which appear to remarkably converge among CSCs and EMT cells, secondary site colonization depends more on a gradual shutdown of cell motility to enable settlement [39]. Therefore, CSCs might be more efficient in metastasis formation since EMT cells first need to undergo the reverse process, termed mesenchymal-to-epithelial transition (MET), before being able to engraft. Thus, CSCs and EMT cells share many similarities at the primary site, but there are considerable differences in their specific requirements at secondary sites, with metastasis formation by EMT cells necessitating a local MET-inducing milieu.

Collectively, CRC progression is driven by both CSCs and EMT cells, which diversify the cellular tumor architecture and impose poor prognosis by mediating metastasis and drug resistance [40, 41]. The role of EpCAM in this tumor-promoting CSC-EMT axis is so far inconclusive and subject to discussion below.

INTERDEPENDENCE OF EPCAM, EMT, AND CSCS

Accumulating evidence suggests a strong and possibly causal link between EMT and the acquisition of stem cell properties in both

normal and malignant epithelial cells [42, 43]. In addition, EpCAM, along with other markers such as E-cadherin and cytokeratins, defines a key denominator of the epithelial cell state. A causal link between EpCAM and EMT was demonstrated in nasopharyngeal carcinoma, where EpCAM regulates EMT through activation of the PI3K/AKT/mTOR pathway [13]. Hence, if EpCAM expression is inversely regulated among colorectal CSCs and EMT cells (high on CSCs and low or lost on EMT cells), EpCAM should be dispensable for the acquisition of malignant traits at the primary site. In contrast, during colonization of secondary sites, the mandatory reacquisition of epithelial properties by disseminated EMT cells indicates that EpCAM might be required for settlement and the subsequent formation of clinically apparent metastasis. This view supports the notion that the tumor-promoting effects of EpCAM are phenotype-dependent and most pronounced in epithelial-like carcinoma cells [44]. Along similar lines, a recent report demonstrated context-dependent regulation of EpCAM expression in early systemic cancer, with high expression correlating to proliferative stages and low expression being associated with invasion and dissemination [45]. This corroborates the hypothesis that clinically apparent outgrowth of metastasis requires EpCAM expression on the disseminated tumor cells. Notably, there is evidence that EpCAM expression counteracts terminal differentiation processes [46], which might be relevant for the promotion and maintenance of a dedifferentiated stem-like state required for long-term tumor propagation.

Another explanation for the complex role of EpCAM in CRC progression and stemness is the contribution of EpCAM protein variants with different functional specificity, such as EpICD and EpCAM^{MT} (EpCAM membrane-truncated). These variants have unique molecular characteristics and are differentially associated with the survival of cancer patients. Mechanistically, nuclear EpICD forms a complex with β-catenin, FHL2, and Lef-1 to regulate oncogenic gene expression [1], with typical target genes being cyclin A and E and the pluripotency-associated transcription factor c-Myc [47]. More recently, our group demonstrated in a large retrospective study that predominant expression of EpCAM^{MT} (indicative of loss of membranous localization of EpICD) correlates with a more aggressive clinical behavior of CRC, resulting in significantly shortened patient survival [5]. Importantly in this study, we observed that truncated EpCAM^{MT} is associated with several factors linked to CSCs and EMT, such as poor differentiation, vascular and marginal invasion, and lymph node metastasis. The question remains whether nuclear EpICD accumulation and the emergence of EpCAM^{MT} represent two sides of the same coin or whether there is independent regulation between the two phenomena. Moreover, what is the particular role of EpICD and EpCAM^{MT} in cancer stemness and EMT?

Since both EpICD and EpCAM^{MT} correlate with aggressive disease and shortened survival, it is conceivable that either variant has a role in conferring cancer stemness, and a possible mechanism underlying this potential ability is c-Myc induction, as described above. Alternatively, the functional properties of EpEX might differ between EpCAM^{MF} (EpCAM membrane full-length) and EpCAM^{MT}, which would indicate direct regulation of EpEX by EpICD. Potential effects include (a) altered function in homotypic cell adhesion mechanistically imposed by different molecular interaction partners and (b) modified downstream signaling affecting oncogenic or stemness-related gene expression. EpICD and EpCAM^{MT} might also participate in the fine-tuning of EMT, which could be characterized by a loss of expression of primarily the full-length form (i.e., EpCAM^{MF}). For instance, nuclear EpICD accumulation, via regulation of gene expression, might be able to induce an EMT-like phenotype that preserves expression of EpCAM^{MT}, thus providing an alternative pathway for EMT independent of surface EpCAM expression. During colonization of secondary sites, rejoining of EpICD and EpCAM^{MT} entailing functional EpCAM^{MF} expression might shut down this pathway to re-establishing the bona fide epithelial cell state and seed metastasis.

In summary, CSCs and EMT are highly correlated and there is great functional convergence. The role of EpCAM in these two phenomena is complex and partially paradoxical, but there is clear interdependence of all players (Fig. 1A). EpCAM variants, such as EpCAM^{MT} and EpICD, can partially explain the broad functional spectrum of EpCAM in cancer and might serve as novel targets for cancer stemness/EMT-depleting intervention (Fig. 1B).

TARGETING EpCAM FOR CSC-DIRECTED CRC TREATMENT

Many studies have focused on EpCAM as a promising target for cancer therapy involving monoclonal and bispecific/trifunctional antibodies, vaccination strategies, or toxin-conjugated antibody fragments. Edrecolomab (17-1A, Panorex), a murine IgG2a anti-EpCAM antibody, was first used in immunotherapeutic treatments of gastrointestinal cancers. Subsequently, treatment with this compound increased the survival of CRC patients in an adjuvant setting in two out of four trials [48]. The drug was approved for the adjuvant treatment of patients with resected CRC in Germany in 1995. However, phase III clinical data were inconclusive and marketing authorization was withdrawn.

Adecatumumab (MT201) is a fully human recombinant monoclonal anti-EpCAM IgG1 antibody that mediates complement-dependent (CDC) and antibody-dependent cellular cytotoxicity (ADCC) with high efficacy. Compared to edrecolomab, this antibody targets a different protein epitope. Its antitumor activity was demonstrated in vivo using a nude mouse model of human cancer [49]. The human nature and the relatively low binding affinity might allow immunological tolerance and confer a favorable safety profile in patients. Adecatumumab was tested in phase II trials in patients with metastatic breast cancer at multiple centers in Europe as well as in early-stage prostate cancer. In metastatic breast cancer, this antibody showed dose- and target-dependent clinical activity, even though no objective tumor regression could be observed [50]. Whether this limitation can be overcome by further tailoring antibody affinity remains to be shown. Nevertheless, it is tempting to speculate that only EpCAM high-expressing patients may benefit from EpCAM-targeted treatment. Accordingly, inadequate selection of patients might be a reason for the low success rates of EpCAM-based antibody treatments so far.

The first anti-EpCAM antibody that has received approval for cancer treatment from the *European Medicines Agency* is catumaxomab (Removab) [51]. Intraperitoneal therapy with catumaxomab in patients with malignant-related ascites was shown to prevent fluid accumulation and efficiently eliminate tumor cells [52]. Treatment with this bispecific, trifunctional antibody (targeting EpCAM and CD3) is approved for patients with malignant ascites derived from EpCAM-positive tumors. However, our group was able to demonstrate that high amounts of soluble EpCAM in malignant ascites may reduce the efficacy of this antibody [53]. Thus, proper patient selection is mandatory to avoid inefficient treatment with catumaxomab.
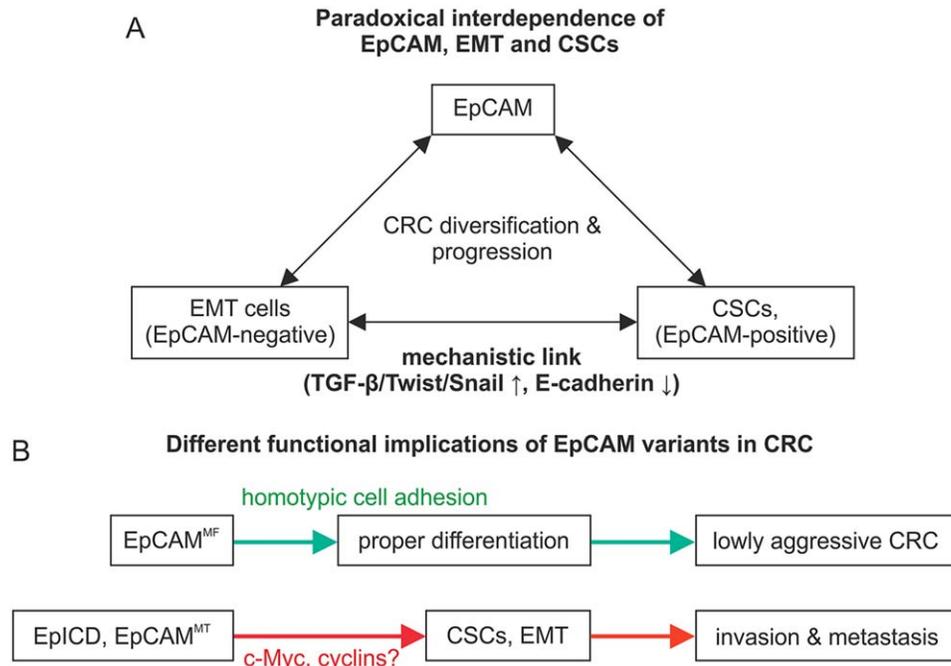
Figure 1. EpCAM variants shape CRC phenotypes. **(A):** Triangular relationship of EpCAM, CSCs, and EMT. CSCs and EMT both accelerate CRC progression and might be mechanistically linked through the indicated (and possibly additional) signaling pathways. The role of EpCAM is somewhat paradoxical as its expression is inversely regulated among CSCs and EMT cells. **(B):** Complexity of EpCAM function in CRC. The full-length form (EpCAM^{MF}) promotes cell adhesion and differentiation, resulting in less aggressive CRC phenotypes. Conversely, nuclear translocation of the intracellular domain (EpICD) and/or predominant expression of the membrane-truncated form (EpCAM^{MT}) lead to cancer cell dedifferentiation that fuels metastatic progression via CSCs and EMT. Abbreviations: CRC, colorectal cancer; CSC, cancer stem cell; EMT, epithelial-to-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; EpICD, EpCAM intracellular domain.

Another bispecific T cell engager (BiTE) class anti-EpCAM/CD3 antibody (MT110) was shown to eliminate colorectal tumor-initiating cells [54]. BiTE antibodies are able to induce target cell elimination by nonactivated peripheral T cells without the need for priming or separate co-stimulation [55]. These molecules are highly stable and induce tumor cell lysis with great efficiency.

Encouraging results were reported for EpAb2–6, another EpCAM-specific antibody [56]. This is the first antibody class compound that induces apoptosis by directly inhibiting EpCAM signaling rather than requiring accessory immune mechanisms for cell killing (ADCC or CDC). EpAb2–6 binds to an epitope localized in the TY loop that is very close to the cleavage site of the ß-secretase BACE1. Binding of EpAb2–6 also inhibits EpICD cleavage, thus reducing nuclear translocation and oncogenic gene activation. In a CRC mouse model, EpAb2–6 was particularly effective in combination with irinotecan. Taken together, this antibody (or others with a similar mode of action) would be ideal candidates for phase I clinical testing in patients stratified according to predominant expression of uncleaved EpCAM (i.e., EpCAM^{MF}).

Although widely expressed on normal epithelia, EpCAM represents an attractive therapeutic target in oncological patients. One explanation for the relatively favorable side effect profile of EpCAM-directed therapeutics is that EpCAM is located on the basolateral side and therefore protected from antibody binding in normal epithelia. During malignant transformation, this polarization is lost and EpCAM expression propagates to the whole membrane. Thus, circulating (micrometastatic) carcinoma cells are ideal targets for EpCAM-directed antibodies. However, potential systemic intolerability of EpCAM-specific immunotoxins or bispecific antibodies as well as pancreatitis with high-affinity antibodies should be considered. Novel EpCAM-directed treatment strategies are under development or in early clinical investigation, and range from immunotoxins [57] to chimeric antigen receptor (CAR)-T cell technology (e.g., clinical trials NCT02915445 and NCT03013712).

While most drug development efforts have so far focused on the generation of antibodies that target the extracellular domain of EpCAM (i.e., EpEX), it is important to emphasize the potent oncogenic activity of EpICD. Nuclear translocation of this factor enables malignancy via the β-catenin/c-Myc pathway such that EpICD can be regarded as a veritable cancer target. Strikingly, both Wnt/β-catenin [58] and c-Myc [59] play a role in conferring cellular stemness; thus, EpICD targeting might even act on the CSCs. Small molecule inhibitors of EpICD (preventing either dissociation from EpEX or translocation to the nucleus) should therefore be envisaged to complement the armada of EpCAM-directed therapeutics. As nuclear EpICD accumulation is quite specific for cancer, small molecule-based treatments might have superior efficacy without doing much harm.

EpCAM-targeted treatments also hold promise for the therapeutic tackling of colorectal CSCs and metastasis. CSCs secure long-term tumor propagation and survive primary therapies owing to their inherent propensity for drug resistance. Colorectal CSCs therefore are an attractive therapeutic target and especially immune-engaging anti-EpCAM antibodies (CDC, ADCC, or BiTE) are an interesting option here. Thus, the adjuvant use of such compounds should be envisaged for the treatment of patients

EpCAM-targeted treatment
-antibodies (EpEX)
-small molecules (EpEX & EpICD)

Bulk tumor (carcinoma)	CSC (EpCAM-positive)	CTC (EpCAM-positive)	MET cell (getting EpCAM-positive)	EMT cell (EpCAM-negative)

| Primary tumor shrinkage | Prevention of metastasis & relapse | Inhibition of hematogenous spread | Inhibition of settlement at secondary sites | **Target missing, no effect** |
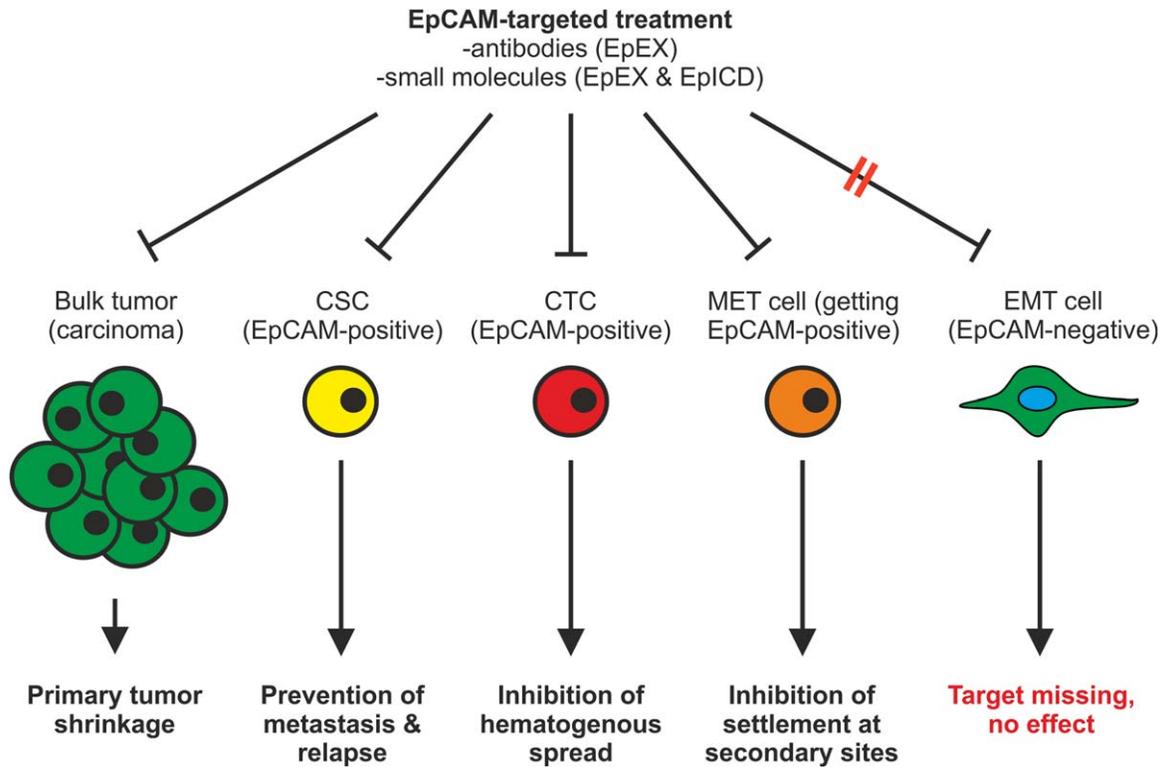
Figure 2. Conceptual framework for therapeutic targeting of EpCAM in malignant disease. Malignant tumors, including those of the colorectum, are characterized by marked heterogeneity and harbor phenotypically and functionally distinct subsets of cells. EpCAM-targeted treatment of colorectal cancer is promising as it should affect many of these subsets including bulk tumor cells, CSCs, CTCs, and MET cells (only EMT cells lacking target expression will be spared). Expected outcomes are the containment (or even shrinkage) of the primary tumor as well as the inhibition of metastatic dissemination and recurrence. Traditionally, EpCAM-directed treatments have focused on antibody-based compounds targeting surface epitopes (i.e., EpEX). The recognition of nuclear translocation of EpICD as a potent oncogenic trigger now asks for complementary approaches involving membrane permeable drugs (i.e., small molecules). Abbreviations: CSC, cancer stem cell; CTC, circulating tumor cell; EMT, epithelial-to-mesenchymal transition; EpCAM, epithelial cell adhesion molecule; EpEX, EpCAM extracellular domain; EpICD, EpCAM intracellular domain; MET, mesenchymal-to-epithelial transition.

with minimal residual disease, or for patients at high risk for recurrence. EpCAM-targeted treatment might also prove beneficial for the prevention of metastasis. EMT tumor cells must reacquire epithelial properties at secondary sites to seed metastasis, and re-expression of EpCAM might be important to increase cell adhesion and establish footholds. When EpCAM-directed treatment is initiated at this particular point in time, the process of settlement might be inhibited thus reducing metastasis formation (Fig. 2). Future studies should hence elaborate on the specific effects of EpCAM-directed therapeutics on colorectal CSCs, metastasis, and circulating tumor cells. This could set the stage for a new paradigm of anti-EpCAM treatment where distinct subsets of cells and specific processes occurring naturally during dissemination and tumor evolution are targeted. A pharmacological challenge here is potential target interaction prior to reaching the metastatic site (neutralization). Dose adaptation, consecutive administration ("conditioning"), and/or optimization of tumor selectivity might therefore be necessary.

CONCLUDING REMARKS

CSCs [33, 34, 60] and EMT [35, 37, 42, 43] both fuel cancer progression and mediate drug resistance. The transmembranous glycoprotein EpCAM exerts distinct and partially paradoxical functions in CSCs and EMT: while CSCs from epithelial-derived

tumors express high levels of EpCAM, EMT cells have downregulated the expression of this key epithelial denominator. The functional convergence of CSCs and EMT despite opposing EpCAM expression argues for context-dependent effects of this molecule and can be at least partially explained by differential contributions from distinct EpCAM variants. In this regard, nuclear signaling of EpICD is an interesting feature that warrants further investigation especially relating to induction of cancer stemness versus EMT. The stage is set to broaden the armada of EpCAM-directed therapeutics and develop specific small molecule inhibitors of EpICD, while further refining the more classic antibody-based approaches. Clearly, the potential of EpCAM-directed therapeutics is great and extends beyond CSCs and bulk cells of the primary tumor (Fig. 2).

AUTHOR CONTRIBUTIONS

M.B.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval

of manuscript, figure design; G.S.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; A.S.: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript.

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