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Epithelial to mesenchymal transition and the cancer stem cell phenotype: Insights from cancer biology with therapeutic implications for colorectal cancer

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Abstract

Although mortality from colorectal cancer (CRC) is decreasing, colorectal cancer is still the second highest cause of cancer related deaths in America. Chemotherapy and radiation therapy now play central roles in our strategies to fight cancer, although we continue to lack novel strategies overcoming therapeutic resistance. Molecular mechanisms of therapeutic resistance in CRC continue to be under intense investigation. In this review, we highlight the recent evidence linking epithelial-to-mesenchymal transition (EMT) with aggressive tumor biology as well as with the cancer stem cells (CSC) across multiple organ systems including colon cancer. Furthermore, in the era of neo-adjuvant treatment, the clinical implications are concerning that our treatments may have the potential to induce more aggressive cancer cells through EMT, perhaps even generating CSCs more capable of metastasis and further resistant to treatment. This concern and potential reality highlights the critical need for further understanding the impact of clinical therapy on the pathobiology of cancer and further supports the need to therapeutically target the CSC. Besides serving as potential biomarkers for aggressive tumor biology and therapeutic resistance, EMT and CSC molecular pathways may highlight novel therapeutic targets as strategies for improving the response to conventional anti-neoplastic agents translating into improved oncologic outcomes.

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Keywords

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Background

As of 2012, colorectal cancer (CRC) remains the third most frequently diagnosed cancer with an estimated 103,000 new cases per year [1]. Although mortality from CRC is decreasing, colorectal cancer is still the second highest cause of cancer related deaths in America [1]. Treatment resistance is unfortunately common even with improved therapeutic strategies. Neoadjuvant chemoradiation has become an accepted approach in patients with locally advanced rectal cancer (LARC), resulting in improved tolerance of prescribed therapy, improved organ preservation, and less treatment related complications [2]. Complete pathologic response rates (no evidence of viable tumor) following pre-operative therapy range between 10–20% [3]. Therefore, resistant tumors are present in up to 90% of the cases with approximately 40% of the cases demonstrating no significant response to conventional therapy [4]. Tumor response as measured by regression grading is an effective surrogate marker of long-term survival and recently was demonstrated as an effective benchmark for oncologic outcomes in LARC patients [5–7]. Improving therapeutic response rates to preoperative therapy should ultimately translate into better outcomes associated with CRC. Given the high rate of resistance, highlighted by the lack of complete response in the majority of rectal cancer patients, exploring novel molecular strategies to enhance conventional therapy for CRC is desperately needed.

Molecular mechanisms of therapeutic resistance in CRC continue to be under intense investigation [8, 9]. Recently, investigations have demonstrated a convincing link between epithelial-to-mesenchymal transition (EMT) and cancer stem cells (CSC) as well as the association of these processes with CRC progression and therapeutic resistance [10, 11]. Therefore, EMT and CSC molecular pathways associated with chemoradiation resistance should provide insight into tumor survival mechanisms and suggest potential novel targets to improve CRC treatment strategies.

Epithelial-to-Mesenchymal Transition in Cancer

EMT is a unique process initially characterized in embryonic development in which cells lose epithelial features and gain mesenchymal properties [12]. EMT results in epithelial cells becoming spindle shaped, with loss of cellular polarity similar to mesenchymal cells. These phenotypic changes of EMT correlate with increased cellular motility and invasion more characteristic of mesenchymal cells [12]. This transformation between two morphologically different states was initially described in 1908 and was first associated with chick development in 1960. This transformation is associated with loss of E-Cadherin in epithelial cells and increased expression of mesenchymal markers, such as Vimentin [13, 14]. Loss of E-Cadherin is a critical step in EMT and corresponds with the morphologic cellular alterations [15, 16].

More recently, molecular pathways associated with EMT have been identified in cancer cells with analogous roles as observed in development (Figure 1) [12]. Growth factors such as hepatocyte growth factor, transforming growth factor-β2, and epidermal growth factor are potent initiators of EMT [17–20]. Activated growth factor receptors lead to intracellular signaling cascades ultimately resulting in the downregulation of E-Cadherin. The signaling cascades activating EMT directly alter the cellular cytoskeletal matrix as well as change gene expression profiles further enhancing transformation [16]. With the loss of E-Cadherin expression, epithelial cells breakdown cell-cell adhesions and become more migratory [16].

The breakthrough discovery of the zinc finger molecule *SNAI1* (snail) in *Drosophila*, a transcriptional repressor of E-Cadherin, provided new information into the molecular mechanisms driving EMT [21]. This discovery uncovered a critical link between intracellular signaling and transcriptional inhibition of E-Cadherin. Signaling pathways activate snail, which binds critical E2 boxes proximal to the transcriptional site of the E-Cadherin promoter, silencing gene expression [13]. Subsequently, other critical E-cadherin transcriptional repressors have been discovered, such as *SNAI2* (slug), zeb1/2, SMAD interacting protein 1 (SIP1), and the basic helix-loop helix family member TWIST1, each having similar functions [12].

Epithelial-to-Mesenchymal Transition and MicroRNAs

MicroRNAs (miRNAs) are small non-coding RNAs that induce mRNA degradation or translational repression through specific base pairing, typically within the 3′ UTR [22, 23]. They have been implicated in the regulation of most cellular processes and, of importance here, their role in the regulation of cancer progression and metastasis and more specifically EMT. For example, miR-9, which is upregulated in breast cancer cells, directly targets E-Cadherin leading to increased cell motility and invasiveness [24]. Overexpression of miR-9 in otherwise non-metastatic breast tumor cells enabled cells to form pulmonary micrometastases in mice. Conversely, inhibiting miR-9 by using a ‘miRNA sponge’ in highly malignant cells inhibited metastasis formation [24]. miR-495 expression in breast cancer cells promoted colony formation in vitro and tumorigenesis in mice [25]. Similar to miR-9, miR-495 promoted cell invasion and oncogenesis via direct suppression of E-Cadherin. Interestingly, investigations have also demonstrated that miRNAs can induce gene expression through promoter binding. Specifically, miR-373 was found to bind and activate the promoter of E-Cadherin, which adds to the complexity by which miRNAs may regulate gene expression [26].

Other hallmark mediators of EMT such as Vimentin and N-Cadherin have also demonstrated regulation by miRNAs. miR-30a was shown to inhibit cell migration and invasion in breast cancer by directly targeting Vimentin. Furthermore, reduced tumor expression of miR-30a in breast cancer patients was associated with an unfavorable outcome, including late tumor stage, lymph node metastasis, and worse outcomes including increased recurrence rates and decreased long-term survival suggesting the utility of miR-30a as a potential breast cancer prognostic marker [27]. Other tumor suppressive microRNAs such as miR-138 and miR-17-3p have also demonstrated an anti-neoplastic effect in part by targeting Vimentin [28, 29]. Specifically, miR-17-3p suppressed Vimentin

expression in prostate cancer and expression of miR-17-3p in prostate cancer tumor specimens and cell lines inversely correlated with aggressiveness [29]. This study showed that expression of miR-17-3p is low in highly tumorigenic, metastatic cell lines, but increased in cell lines that display decreased tumorigenicity. As well, miR-17-3p expression was also inversely associated with increased prostate cancer Gleason Score. Finally, miR-17-3p restoration blocked tumor growth in male athymic, nude mice supporting their hypothesis that miR-17-3p may function as a tumor suppressor in prostate cancer [29].

N-Cadherin is also regulated by microRNAs as well through 3′ UTR negative regulation. miR-145 was shown to suppress gastric cancer cell migration and invasion *in vitro* through direct targeting of N-Cadherin [30]. This study further showed miR-145 inhibited experimental metastasis *in vivo* confirming its function in suppressing the invasion-metastasis cascade. Similarly, miR-194 targets the 3′-UTRs of several genes involved in EMT and cancer metastasis, including N-Cadherin [31]. EMT regulating transcriptional factors have also been identified as targets of specific miRNAs. These include snail by the miR-30 family [32], slug by miR-124 [33] and zeb1 & 2 by the miR-200 family [34–37]. The growing body evidence demonstrates how miRNAs impact multiple levels and mediators involved in tumor plasticity and EMT highlighting their significance and the importance of expanding our understanding these complex interactions [24, 34, 38]. Another important point that is an area for future studies is that miRNAs have multiple gene targets perhaps compounding the downstream effect [39–41].

Epithelial-to-mesenchymal transition and Therapeutic Resistance

Besides the well-described relationship between EMT and enhanced motility, recently, mediators of EMT have been associated with enhanced cellular survival [42, 43]. Snail expression in Madin-Darby canine kidney cells attenuated cell death in response to serum starvation and TNF-α treatment. The anti-apoptotic response as a result of snail expression was associated with activation of both MAPK and PI3K pathways [42]. Similarly, transfection of slug into MCF7 breast cancer cells promoted resistance to DNA damage-mediated programmed cell death via inhibiting multiple pro-apoptotic factors including p53, DNA Fragmentation Factor 40 (DFF40), and BH3 Interacting Domain Death Agonist (BID) [43]. The association of EMT with enhanced survival pathways and resistance to apoptosis has fueled interest in exploring the link with resistance to anti-neoplastic therapeutic strategies.

Recent investigations have also demonstrated an association between acquired chemotherapy resistance in cancer cells with an upregulation of EMT molecular alterations in gastrointestinal malignancies [9, 10]. In pancreatic cancer cell lines, acquired gemcitabine resistance demonstrated alterations in their phenotype consistent with EMT [44]. The resistant pancreatic cancer cell lines demonstrated loss of E-Cadherin expression, β-catenin nuclear translocation, and increased Vimentin levels. Similarly, in CRC cells, chronic oxaliplatin exposure resulted in resistant cells that displayed the phenotypic alterations associated with EMT such as loss of polarity, spindle shape, and increased mobility [9]. The oxaliplatin-resistant cells also demonstrated decreased E-Cadherin expression, as well as increased snail and Vimentin expression, hallmark molecular changes associated with EMT [9]. These studies suggest that cancer cells in response to cellular stress induced by chemotherapy undergo

EMT as an adaptive mechanism resulting in an anti-apoptotic and pro-survival state. Alternatively, rather than chemotherapy inducing EMT, chemotherapy may result in clonal selection and propagation of cells with enhanced pro-survival pathway activation as observed with EMT.

EMT pathways have also been directly implicated as mediators of intrinsic chemotherapy resistance [11, 45]. A study demonstrated that snail-transfected Panc-1 cancer cells developed increased EMT properties as well as decreased sensitivity to both 5-FU and gemcitabine chemotherapy treatment [45]. More recently, forced Snail expression in colon cancer cells enhanced the CSC phenotype and also oxaliplatin resistance, thus demonstrating that EMT mediators directly lead to therapeutic resistance [11]. In this study, snail expressing HCT116 and HT29 cells demonstrated morphological, functional, and molecular properties consistent with EMT as well as an ~10-fold greater resistance to oxaliplatin compared with control cells.

Although molecular therapies targeting VEGF (vascular endothelial growth factor) and EGFR (epidermal growth factor receptor) have demonstrated success in patients with gastrointestinal malignancies, appropriate patient selection and the eventual development of therapeutic resistance are challenges limiting progress [8, 46, 47]. Studies performed in head and neck squamous cell carcinoma (HNSCC) and lung cancer have highlighted the role of EMT in determining outcomes following molecular-targeted therapy and may provide insight for the future management of gastrointestinal malignancies [48–50]. In HNSCC, erlotinib (a tyrosine kinase inhibitor of EGFR) sensitivity was determined in a panel of 42 cancer cell lines [50]. Based on microarray analysis and Western Blot analysis, erlotinib resistance corresponded with increased Vimentin and loss of E-Cadherin, claudin-4 and claudin-7 expression. This pattern of protein expression reflects hallmark changes associated with EMT. To investigate the clinical relevance of EMT, the investigators analyzed tumor specimens from subjects previously enrolled in a randomized trial in which erlotinib failed to demonstrate clinical activity [50]. Interestingly, in E-Cadherin negative tumors, time to progression following erlontinib treatment was significantly shorter relative to E-Cadherin positive tumors. Furthermore, in patients with non-small cell lung cancer treated with gefitinib (another tyrosine kinase inhibitor of EGFR), increased E-Cadherin immunohistochemical staining was associated with longer time to progression compared to tumors with low E-Cadherin staining (12.4 vs. 5.9 months) as well as improved overall survival (18.4 vs 13 months) [49]. Similar to what has been observed in studies exploring acquired chemotherapy resistance, chronic exposure to bevacizumab (Avastin; an angiogenesis inhibitor targeting VEGF) significantly enhanced migration and invasion of multiple colon cancer cell lines associated with increased activation of VEGF receptor 1 [51]. These recent discoveries implicating EMT in chemoresistance in a variety of cancer highlights the importance of developing novel therapies targeting EMT pathways to improve oncologic outcomes perhaps complimenting conventional therapies including chemotherapy and radiation.

Cancer Stem Cell Theory

Vogelstein first described the evolution of colon cancer from normal epithelium to pre-malignant adenomatous tumors that ultimately progress to adenocarcinoma [52]. This model attributes cancer progression to result from clonal evolution due to sequential molecular mutations leading to uncontrolled growth, invasion, and metastasis. The underlying assumption follows a stochastic model in which tumor cells acquire the same potential for growth and propagation. Conversely, the CSC theory has gained enthusiasm with growing evidence supporting the concept that small populations of cancer cells represent the tumor-initiating cells [53]. The stem cell concept is centered on a hierarchical theory for cancer development suggesting that only specific undifferentiated cancer cells, the tumor-initiating cells, have the ability to self-renew, propagate and differentiate leading to cancer growth and progression [53]. This hierarchical model separates CSCs with the ability to propagate tumor development from non-stem cells that have lost their tumorigenic potential and is supported by evidence from both hematologic and solid organ malignancies [54–56]. Both the stochastic and hierarchical models of cancer propagation are supported by the fundamental concept of clonal evolution [57]. This theory was supported by a recent study in CRC where single-cell cloned CRC CSC could self-renew and were capable of multilineage differentiation in vivo [58].

CSCs were initially identified in acute myeloid leukemia with the isolation of CD34+/CD38− populations of leukemia cells with tumor initiating properties [54]. Subsequently, CSCs have been identified in solid organ malignancies using similar strategies first in breast cancer followed by the description of CSCs in a variety of cancers including brain, pancreatic, and colon cancer [55, 56, 59–61]. Hallmarks of CSCs include the ability to form tumors in immunodeficient mice by xenograft transplantation and tumorsphere formation in non-adherent 3D cultures [54–56, 62]. When transplanted, as few as 100 isolated CSCs can successfully develop tumors in xenograft models [59]. CSCs have other aggressive features such as the ability to mediate angiogenesis and demonstrate resistance to apoptosis [63]. The presence of CSCs in human tumor specimens has correlated with poor prognosis across many organ systems including CRC [64–66].

Appropriate cell surface markers used for identification and isolation of CSCs varies based on the organ studied, remains controversial and continue to be defined further. For example, breast CSCs are commonly identified by demonstrating a CD44+/CD24− expression pattern [59]. However, pancreatic CSC are most accurately identified as CD44+/CD24+ as well as a third marker such as epithelial cell adhesion molecule (ESA). In a landmark publication, CD44+/CD24+/ESA+ pancreatic cancer cells were highly tumorigenic able to form tumors with implantation of only 100 cells [61]. The most widely studied CSC marker is CD133 (also known as AC133) a 120kDA transmembrane protein. Based on CD133 expression, colon CSCs were first identified representing a small fraction <3% of the individual tumors sampled [9, 55]. Besides CD133, other surface antigens have been used to identify colon CSCs such as EpCAM, CD44, and CD166 [67]. Several reports have demonstrated co-localization of CD133/ CD44 and suggest that this combination may be the most effective CRC specific CSC identification marker [67, 68].

Cancer Stem Cells and MicroRNAs

Evidence is growing highlighting the complex pathways that regulate CSC and stem-like properties in cancer cells. Recently, miRNAs have been demonstrated to directly target transcription factors involved in the promotion of CSCs, or 'stemness' transcription factors. For example, miR-145 is low in self-renewing human embryonic stem cells (hESCs) but highly upregulated during differentiation and was shown to directly target the transcription factors Oct4 (octamer-binding transcription factor 4), Sox2 (SRY (sex determining region Y)-box 2), and Klf4 (Krüppel-like factor 4) [69]. miR-29 directly targets Klf4, a transcription factor required for the reprogramming of differentiated cells to pluripotent stem cells, and for the maintenance of breast cancer stem cells. Downregulation of miR-29 members potentiates the expansion of cytokeratin 5(+) and CD44(+) cells in response to progestins, and results in increased stem-like properties *in vitro* and *in vivo* [70].

Myc is a transcription factor that is well known for its role in cancer progression. A mutated version of Myc is found in many cancers, which causes Myc to be constitutively (persistently) expressed. This leads to the unregulated increased expression of various genes promoting cancer formation. Furthermore, Myc has been identified as an essential factor driving the production of pluripotent stem cells and has been linked with putative ovarian CSC factors including Oct4 and Nanog [71, 72]. Recently, the microRNA let-7a was demonstrated to negatively regulate Myc resulting in cell growth/ proliferation inhibition in lymphoma cells [73]. Along with let-7a, other miRNAs have demonstrated the ability to regulate Myc expression adding to the complexity of Myc regulation and suggest that dysregulation of these miRNAs participates in the genesis and maintenance of the lymphoma phenotype in Burkitt lymphoma cells and other Myc-dysregulated cancers [74–76].

In CRC, miR-451 was downregulated in colonosphere forming cells compared with the parental cell lines. In contrast, the expression of miR-451 in CRC cells decreased CSC properties as well as increased sensitivity to Irinotecan suggesting an inverse association between miR-451 and CSC. [77]. Furthermore, the expression of miR-451 also correlated with CRC patient response to Irinotecan. These examples highlight the complex regulatory pathways involving microRNAs and CSC that can be potentially taken advantage of in designing the next generation of molecular targeted therapies.

Cancer Stem Cells and Therapeutic Resistance

The CSC model of cancer evolution has critical implications to modern cancer therapeutics. The CSC model offers both an explanation for cancer therapeutic failure and highlights critical new strategies developed to specifically target this subpopulation of cancer cells to improve future patient survival. Modern chemotherapy strategically targets rapidly dividing cells as opposed to the relatively quiescent CSC. This phenomenon would explain hypothetically why primary tumors might initially respond to therapy and subsequently quickly progress, as well as the observation that recurrences following tumor eradication may reappear years following therapy. Unique protection mechanisms have been reported associated with CSCs besides quiescence including: high expression of ATP-binding cassette transporter protein, enhanced DNA repair mechanisms and resistance to DNA

damage, and expression of anti-apoptotic proteins [78–82]. Breast CSCs have demonstrated lower levels of reactive oxygen species due to enhanced expression of free radical scavenger activity in response to radiation [83]. In a recent report supporting the association between CSCs and therapy resistance, a chemoresistant population of CRC cells demonstrated CSC markers and phenotype [84].

Studies across a range of malignancies have demonstrated that CSCs are enriched following either chemotherapy or radiation therapy [83, 85, 86]. An investigation in breast cancer patients receiving neoadjuvant therapy highlighted the clinical significance of therapeutic resistant CSCs [86]. In this study, residual tumors following neoadjuvant chemotherapy demonstrated increased $CD44^{high}/CD24^{low}$ breast CSCs expression 3-fold compared with pre-treatment levels. In CRC, xenogeneic tumors consisting of CSCs isolated from patient samples based on expression of epithelial-specific antigen (ESA, EpCam) and CD44 demonstrated resistance to chemotherapy [87]. In the chemotherapy treated tumors, residual tumors demonstrated an enriched population of CSCs that were highly tumorigenic [87]. Gene expression patterns from clinical patient samples have also highlighted the association of therapeutic resistance and CSCs. In a study of surgically resected high risk Stage 2 and Stage 3 CRC patients, a stem cell gene expression (66 genes investigated) pattern predicted relapse-free survival with a high stem cell gene profile decreasing relapse-free survival by ~50% [88]. A CSC gene profile has also been associated with poor prognosis following neoadjuvant chemoradiation for rectal cancer. The presence of stem cell markers CD133, Oct4 and Sox2 in post-chemoradiation patient samples predicted distant relapse [89]. Ineffective targeting of CSCs by conventional therapies may be a fundamental reason why cancer therapeutics fail to cure the majority of patients. In the future, developing therapeutic strategies both for CSCs and the remaining tumor population may enhance cancer response rates, translating into better oncologic outcomes (Figure 2).

Cancer Stem Cell, Epithelial-to-Mesenchymal Transition and MicroRNAs

CSCs display aggressive characteristics including increased invasion, metastatic ability and resistance to therapy and predict poor patient prognosis as highlighted above. Extending this concept further, investigators have explored the association between expression of EMT-associated genes with CSC properties across multiple organ systems including colon and breast cancer [11, 90, 91]. Recently, a strong association between EMT-associated gene expression and CSCs has been demonstrated [90, 92]. Both TGF-β treatment and expression of EMT transcriptional mediators twist or snail in human immortalized mammary epithelial cells demonstrated a CD44+/CD24− expression pattern associated with stem cells and a 30-fold enhanced mammosphere formation compared with control cells [90]. In separate investigations, CD44+/CD24− breast cancer cells consistently had higher EMT gene expression patterns than more differentiated tumor cells [91]. Furthermore, forced expression of snail in CRC cell lines resulted in therapeutic resistance and an enhanced CSC phenotype including enhanced spheroid formation as well as increased CD133 and CD44 expression [11].

Supporting the molecular link between CSC and EMT is the recently described negative feedback loop between the EMT transcriptional factors and the microRNA (miRNA) 200 family which are strong epithelial differentiation inducers (Figure 3) [93, 94]. In both

pancreatic and CRC cells, zeb1 demonstrated the ability to repress miR-200 family members including miR-141, 200c and 203 [93, 94]. Conversely, the miR-200 family members were able to inhibit zeb1 and Bmi1 expression providing a negative feedback loop [93, 94]. Similarly, breast CSCs demonstrate low expression of microRNA 200 family including mirR-200c that negatively regulates the EMT transcriptional factors zeb1 and zeb2 [95]. In a separate investigation using an inducible breast CSC model, a genetic screen identified miRNAs that inhibited CSC growth and were also downregulated in CSCs [96]. This study confirmed the negative feedback loop between CSCs and miR-200 family and also identified miR-15/16, miR-103/107 and miR-145 as negative regulators of CSCs [96]. Other examples of overlap between miRNAs involved in the regulation of EMT and CSCs exists. One such example includes the study that showed miR-495 is upregulated in breast cancer stem cells and is able to negatively regulate through direct targeting of E-cadherin [25]. These observations relating EMT to CSCs support the finding that CSCs have increased invasiveness and metastatic potential. The association of CSCs with EMT may further highlight critical pathophysiologic CSC pathways and identify novel CSC-targeted therapeutic strategies.

Cancer Stem Cell Directed Therapies in Clinical Development

With the growing evidence implicating CSCs in therapeutic resistance, clinical strategies continue to be developed specifically targeting CSCs to improve outcomes for cancer patients. Although most experience with targeting CSC is currently pre-clinical, novel CSC-targeted agents have entered clinical use. CSCs are difficult to target directly especially because they represent such a small number of the actually cancer tumor burden. Strategies specifically targeted CSC cell surface markers such as CD44 have been explored in leukemia as well as breast cancer with success demonstrated in pre-clinical models [97, 98]. Alternatively, drugs such as salinomycin have been identified that are toxic to breast CSCs through unbiased small molecule screens and represent a potential CSC specific therapy [99]. Similarly, metformin has been identified to have CSC specific cytotoxicity and has demonstrated the ability to enhance the effects of chemotherapy in a range of cancers [100, 101].

Agents targeting CSC unique mediators have demonstrated pre-clinical success and have already been used in clinical trials [102]. For example, ATP-driven efflux pump inhibitors such as Dofequidar fumarate have demonstrated clinical efficacy in breast cancer. In a randomized clinical trial, Dofequidar fumarate (MS-209) improved the efficacy of cytotoxic chemotherapy in recurrent and advanced breast cancer [102]. Another similar drug, tariquidar has been explored in conjunction with docetaxel for patients with lung, ovarian, and cervical cancer [103]. Various other molecular pathways targeting CSCs are under investigation including the Hedgehog pathway, Notch signaling, and CXCR4-CXCL12 [104–106]. As our understanding of the CSC phenotype and critical pathways driving CSC survival continue to improve, our therapeutic CSC targeted option should continue to grow potentially impacting all cancer subtypes.

Conclusion

In the evolution of cancer, the dual processes of EMT and CSCs in survival and mobility may compliment one another, enhancing the aggressiveness of tumor cells. As a mediator of survival, EMT and CSCs protect cancer cells from hostile environments and allows cells to escape to distant sites more conducive for survival. In the era of neo-adjuvant treatment for CRC, the clinical implications are concerning that our treatments may actually contribute to the development of more aggressive cancer cells, perhaps even generating CSCs more capable of metastasis and further resistant to treatment. This concern and potential reality highlights the critical need for further understanding the impact of clinical therapy on the pathobiology of cancer and further supports the need to therapeutically target the CSC.

In summary, chemotherapy and radiation therapy now play central roles in our strategies to fight cancer, although we continue to lack novel strategies overcoming therapeutic resistance. We now understand that the dynamic process of EMT serves to enhance tumor progression by increasing cellular mobility and improving cellular survival. Evidence now exists which links EMT with aggressive tumor biology as well as with the CSCs across multiple organ systems including colon cancer. Besides serving as potential biomarkers for aggressive tumor biology and therapeutic resistance, EMT and CSC molecular pathways may highlight novel therapeutic targets as strategies for improving the response to conventional anti-neoplastic agents translating into improved oncologic outcomes.

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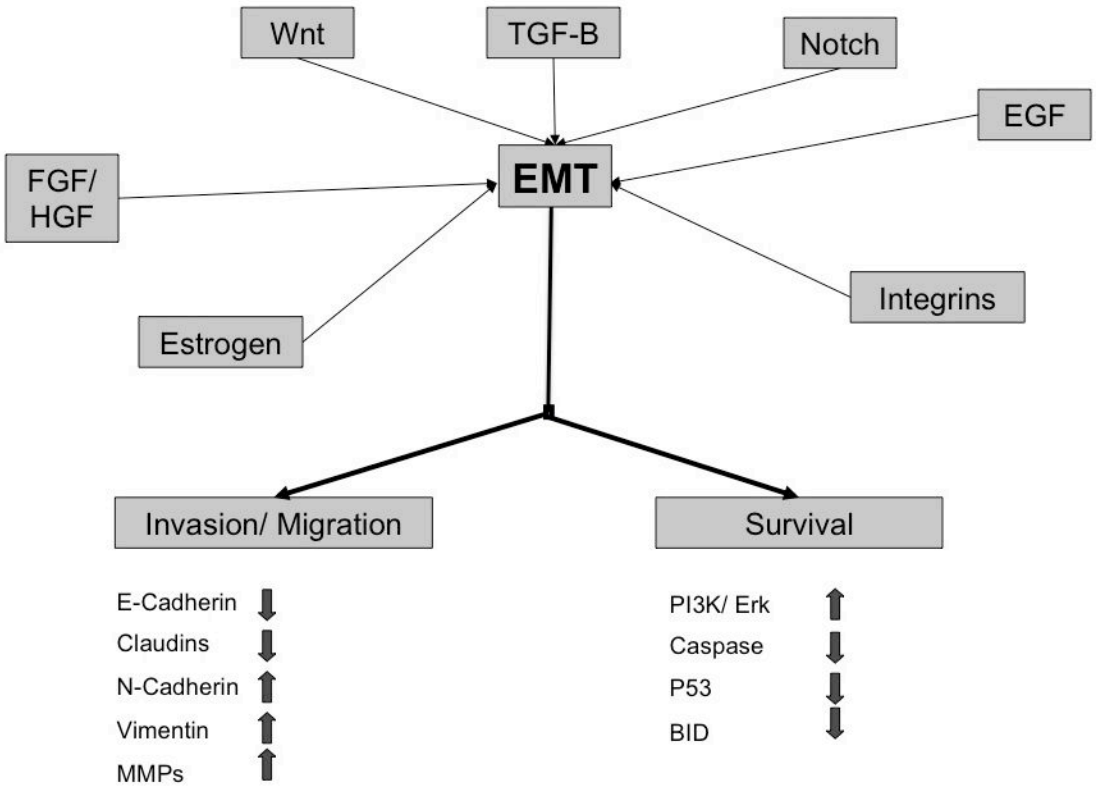

Figure 1. EMT pathways deregulated in cancer and the downstream effects

The diagram demonstrates the variety of effector pathways for EMT as well as the downstream consequences related to activation of the classic EMT transcriptional mediators. Abbreviations: TGF-b: transforming growth factor beta; EGF: Epidermal growth factor; HGF; hepatocyte growth factor; FGF: fibroblast growth factor; MMP: Matrix metalloproteinase; BID: BH3 interacting-domain.

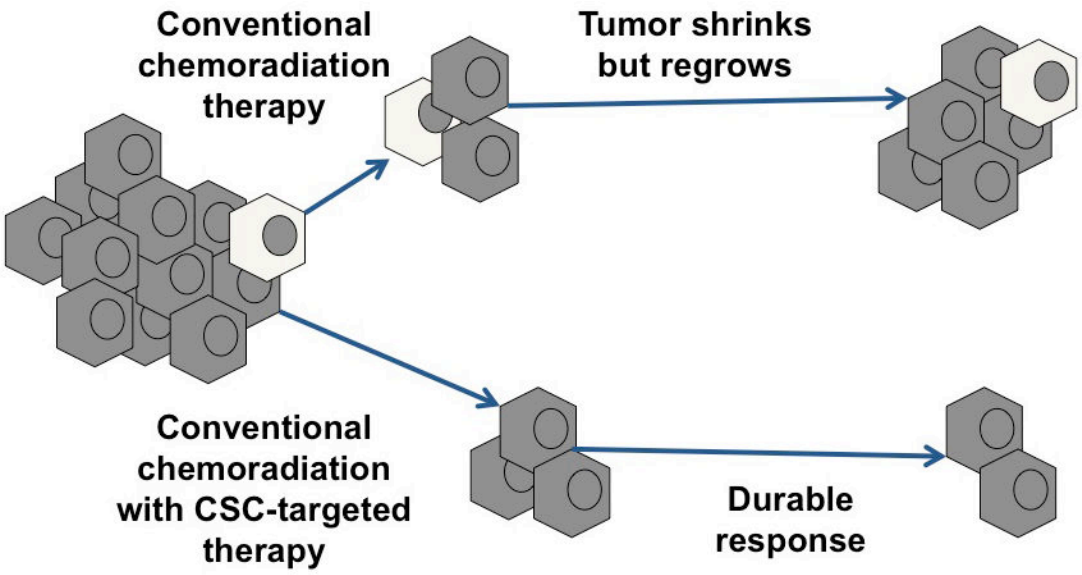

Figure 2. Cancer stem cell therapeutic rationale

The upper pathway demonstrates the failure of conventional therapy in the presence of cancer stem cells that are resistant to therapy with subsequence tumor regrowth. The lower pathway demonstrates the potential of successful stem cell therapy resulting in a durable clinical response. Abbreviation: cancer stem cell (CSC)

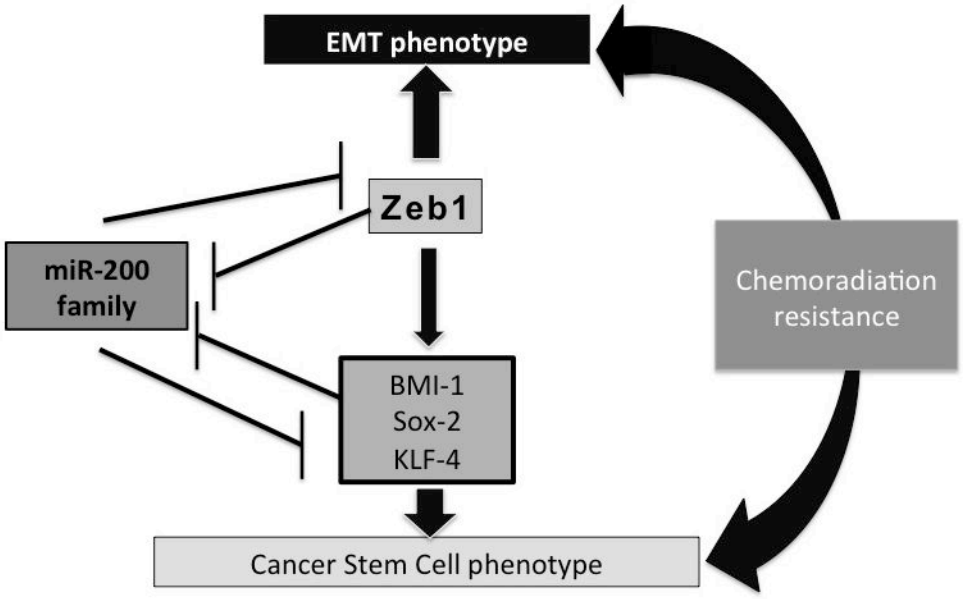

Figure 3. The negative feedback loop between transcriptional mediators of cancer stem cells and EMT with the miR200 family

The diagram demonstrates the growing understanding of the complex pathways involved in miRNA signaling specifically highlighting the negative feedback loop between miRNA-200 family, EMT, and CSC mediators. Abbreviations: BMI-1: B lymphoma Mo-MLV insertion region 1 homolog; Sox-2: sex determining region Y)-box 2; KLF-4: Kruppel-like factor 4.