The Interplay Between EMT and Cancer Stem Cells in Tumor Expansion and Promising Antitumor Strategies

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Abstract

The statistically significant correlation between cancer-related deaths and metastasis makes tumor metastasis prevention crucial in the improvement of therapeutic and clinical treatments. With its ability to confer cancer cells more mobility, invasive capability, and resistance to apoptosis, the epithelial-mesenchymal transition (EMT) has been suspected for its role in driving cancer progression from carcinogenesis to metastasis. More importantly, tumor cells, after undergoing the EMT, have shown acquired traits of stem cells, which makes them even stronger in therapeutic resistance. Scientists are increasingly targeting the EMT pathways as their complex biological steps have been seen as promising opportunities to find cancer treatments or even cures. However, experiments testing the EMT's influence on metastasis *in vivo* have been technically challenging and generated unexpected results. So, having a clear-cut definition of and understanding how the EMT worsens cancer metastasis remains an unachieved mission. To accomplish this mission, many studies have started to use *in vivo* imaging, advanced lineage tracing systems, and *in vivo* models. These tools could efficiently help uncover the intricate driving mechanism of EMT in metastasis. This review discusses the recent advances scientists have made regarding the biological concepts of EMT in boosting metastasis and future clinical or therapeutic innovations.

Keywords: Biology; Cancer Development; Cancer Stem Cells, Epithelial-Mesenchymal Transition

Introduction

First observed in the development of embryos, the EMT is a process in which cells lose their epithelial features and gain mesenchymal features.[1] EMT results in spindle-shaped cells that have removed cellular polarity.[1] These traits are often characterised by mesenchymal cells which have more motility and invasive tendencies.[1] This transition is a transformation between two morphologically different states and types of cells.[1] The epithelial cells lose their E-cadherin and adopt more vimentin, a mesenchymal cell marker.[2] The loss of E-cadherin in epithelial cells is a fundamental process in the EMT because it triggers a cascade of morphologic alterations that allow for a full transition.[2] More notably, cancer cells have shown expression of EMT-related molecular pathways that plays the same role in embryonic development.[3]

Many growth factors, including hepatocyte, transforming growth factor-β2, and epidermal growth factor, are key EMT initiators.[4] When these factors are activated, intracellular signaling cascades are triggered to downregulate E-cadherin.[4] Besides, the signaling cascades also alter the cellular cytoskeletal matrix and certain pattern of gene expressions, all leading to a clear-cut transformation.[4] After epithelial cells lose their E-cadherin, their cell-cell adhesions also break down, making it easier for them to migrate.[4] A transcriptional repressor of E-cadherin called SNAI1 (snail) is one key element to understanding driving mechanisms of EMT.[5]

The discovery of this zinc finger molecule provides opportunities for investigation of the link between intracellular signaling and downregulation of E-cadherin.[5] Signaling pathways activating SNAI1 silence the gene expression of E-cadherin through binding the critical E2 boxes to the transcriptional site of the E-cadherin promoter.[6] Alternatively, SNAI2 (slug), zeb1, zeb2, SMAD-interacting protein 1, and TWIST1 are other E-cadherin transcriptional repressors that have been discovered that also serve similar functions to SNAI1.[7]

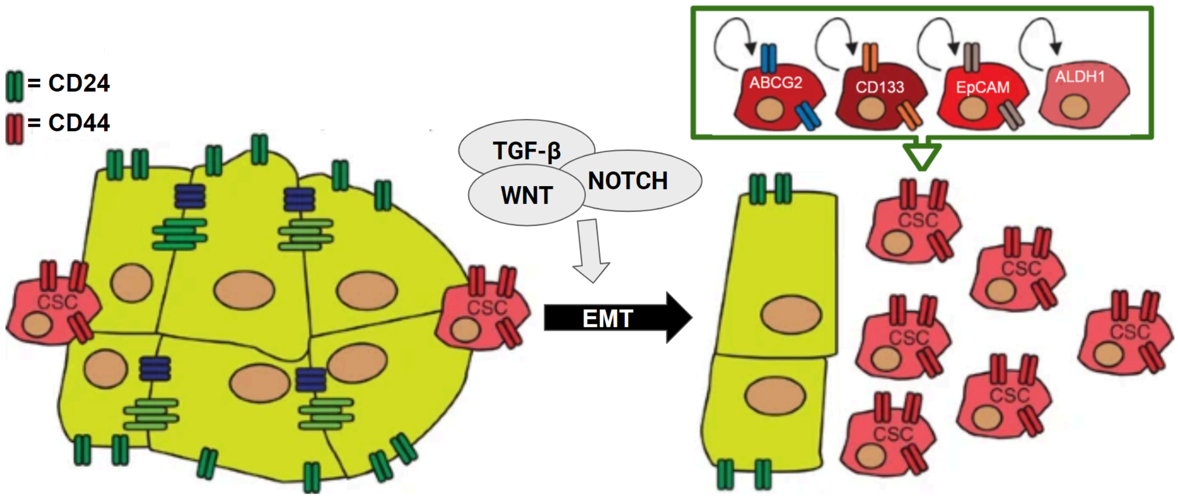

Figure 1: The ability to initiate and expand a tumor has long been known as a unique hallmark of cancer stem cells (CSCs), otherwise regarded as the source of cancer progression.[8] Scientists can use a set of marker proteins such as ABCG2 (a member of the ABC family transporter), CD133, EpCAM (an epithelial cell adhesion molecule), and ALDH1 (aldehyde dehydrogenase 1), to mark the cancer stem cells, so that they could be separated from the other cancer cells.[9] Cancer stem cells can both indefinitely self-renew, which ensures the long-term survival of cancer cells, and differentiate, which ensures that the tumor heterogeneity is maintained, making treatments less effective.[9] The EMT can induce a CSC-like phenotype to original cancer cells.[10] TGF-β, Wnt or Notch are examples of EMT inducers that result in cells acquiring a CD44 high CD24 low phenotype, which closely resembles that of CSCs.[11]

Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is a process, as the name implies, in which epithelial cells turn into mesenchymal cells through the acquisition of certain characteristics.[12] This EMT process can be observed in many biological processes such as tissue fibrosis, embryonic evolution, tissue formation, and wound healing.[12] Moreover, EMT can be key in tumor growth, drug resistance, and metastasis.[13] Since this transition plays a major role in cancer development, it has been targeted in many therapeutic and clinical treatments.[13] The EMT is a result of the synergization of many different signaling pathways such as the transforming growth factor beta (TGF-β) signaling pathway, the receptor tyrosine kinase (RTK) signaling pathway, the Wnt/β-catenin signaling pathway, the signal transducer and activator of transcription 3 (STAT3) signaling pathway, the extracellular matrix (ECM)-mediated signaling pathway, and many more.[13] Many of these signaling pathways initiate EMT by mediating the three main transcription factors: Snail, Twist, and ZEB.[14] Additionally, these pathways also upregulate mesenchymal cell markers, downregulate epithelial cell markers, and thus alter the overall property of epithelial cells.

Snails are members of the family of the ZINC finger transcription factors.[5] There are three types of Snails: Snail1 (Snail), Snail2 (Slug), and Snail3 (Smuc).[5] Out of the three, Snail1 has a significant role in the modulation of cancer cell migration and metastasis through the epithelial-mesenchymal transition.[5]

Many different Snail1 transcription factors can bind to promoters to activate expression of metastasis-associated 1 family member 3 (MTA3), hypoxia-inducible factor 1-alpha (HIF-1a), glioma-associated oncogene homolog 1 (Gli1), lysyl oxidase-like 2 (LOXL2), and many more.[15] Besides, many of these factors can bind to the E-cadherin gene, the gene responsible for alterations of E-cadherin. The sudden shifts in expressed E-cadherin levels are strongly correlated with EMT and cancer metastasis.[16] Also, Snail1 can regulate proteins involved in extracellular cell-cell interaction and intracellular signaling pathways such as Claudin (CLDN), Occludin (OCLN), Zona occludens 1 (ZO-1), Cytokeratin 18, and Mucin 1.[17] Some discoveries have even proven that Snail1 modulates the expression of two types of matrix metalloproteinases (MMP): MMP-2 and MMP-9.[18] More importantly, Snail1 mediates the gene expression of other transcription factors involved in enhancing EMT, namely ZEB-1 and ZEB-2.[19]

The second main transcription factor in EMT is called Twist, which is classified as a BASIC HELIX-LOOP-HELIX (BHLH) and often plays many key roles in many physiological pathways.[20] There are two types of Twist, Twist-1 (Twist) and Twist-2 (Dermo-1), which help cells form their mesodermal layer.[21] Mutated Twist in drosophila causes a mutated phenotype in which they lack internal organs.[22] This Twist mutation, in humans, can lead to Saethre-Chotzen syndrome, where patients have craniosynostosis and mild limb anomalies.[22] Twist-1 plays a pivotal role in many progressive steps that result in cancer metastasis, angiogenesis, and stemness.[23] There is a high correlation between Twist-1 and Twist-2 expression and cancer cell properties, including frequent invasion, migration, and anoikis resistance.[24,25] Therefore, increased Twist-1 and Twist-2 expression can theoretically aid the EMT process, which aids tumor metastasis.[24,25]

The third key transcription factor of EMT is Zinc finger E-box-binding homeobox ZEB.[26] These factors, affecting gene expression of many proteins that affect embryogenesis, differentiation, tumorigenesis, and metastasis, can be found in two forms: ZEB1 and ZEB2.[26] ZEB1 and ZEB2 are fundamental to the EMT process, because they restrict E-cadherin expression by binding to the E-cadherin promoters' E-BOX sequences.[27] When E-cadherin is downregulated, cancer cells get induced traits of mesenchymal cells, which will then metastasize.[27] ZEB1, by binding to the promoter of other genes that makes important proteins in cell-cell interaction, tight junctions (TJ), desmosomes, and cell polarity, reduces epithelial cell properties and enhances metastasis.[28]

Cancer cells, undergoing the EMT process, often express anoikis resistance.[29] Anoikis is simply the programmed cell death (apoptosis) in which epithelial cells self-degrade from breaking their extracellular matrix and neighboring cells.[30] Anoikis is a process that could prevent metastasis.[30] However, cancer cells, wanting to metastasize, tend to avoid anoikis by separating from the original tumor and hitchhiking around the body through the circulatory and lymphatic system.[31] Many EMT-promoting proteins are responsible for cancer cells' anoikis resistance.[32] These EMT-promoting proteins decrease E-cadherin expression, increase N-cadherin expression, and therefore further strengthen anoikis resistance.[33] Similarly, Twist, Snail, and Zeb1 (the three main EMT transcription factors) also alter the expression of E-cadherin and N-cadherin, thereby enhancing anoikis resistance and metastasis.[33]

Ankyrin-G protein is another important factor in regulating E-cadherin, because it uses E-cadherin as a bridge from the cytoskeleton to the cell membrane.[34] Ankyrin-G protein pushes the NRAGE protein, often found in the plasma membranes, to migrate to the nucleus.[35] In the EMT process, which has low levels of E-cadmium and ankyrin-G, the NRAGE protein is translocated to the nucleus and reduces transcription of the tumor suppressor p14ARF gene.[36]

This directly induces anoikis resistance, because the cells now produce fewer tumor suppressor proteins.[36] All of this shows that the Ankyrin-G protein plays a critical role in maintaining the cancer cells' induced anoikis by hemophilically binding to two neighboring cells. On the other hand, altered YAP phosphorylation of E-cadherin and β-catenin further supports anoikis resistance.[37] N-cadherin activated by the Akt or the PI3K/Akt signaling pathway mediates tumor anoikis resistance.[38]

The EMT has attracted many researchers' attention as the power it holds in regulating cancer cell metastasis is a potential target. For example, simvastatin, a drug treating hyperlipidemia, has inhibited EMT.[39] Simvastatin works by preventing the expression of EMT factors such as cadherin, vimentin, and β-catenin.[39] Therefore, simvastatin can successfully prevent cancer metastasis.[39] Many different Phase II clinical trials have utilized simvastatin to treat advanced-stage carcinomas.[40] Alternatively, LY2157299 (galunisertib) also prevents cancer metastasis by inhibiting the TGF - β pathway, one of the main inducing pathways of EMT.[41]

Cancer stem cells are a vital component of tumors as their mechanism ensures cancer survival and progression.[42] This EMT process primarily leads to stemness in cancer cells, a state in which cancer cells can self-renew and generate many differentiated cells.[42] The stem cells' interaction with the environment is also fundamental to their growth and proliferation.[42] Stem cells utilize these properties for the maintenance of tissue homeostasis. Cancer stem cells use these features to survive and advance their malignancy. Cancer cells are usually killed at an early stage using chemotherapy or radiation, whereas certain tumor cells, namely cancer stem cells, might cause tumor relapse.[43] Cancer stem cells are extremely treatment-resistant and express certain traits similar to stem cells.[43] These cancer stem cells allow for tumor growth, because they give rise to other cancer cells.[43] In short, they are the driving cells behind tumor growth and development. CSCs can be identified in many tumors including liver, breast, prostate, pancreas, leukemia, melanoma, and many more by identification of certain cell surface markers.[44] Frequently used CSC surface markers such as CD24, CD29, CD44, CD90, CD133, epithelial-specific antigen (ESA), and aldehyde dehydrogenase 1 (ALDH1) can be used to separate the smaller population of cancer stem cells, which will be eventually targeted.[45]

EMT-induced Cancer Stem Cells

Rare tumor cells (cancer stem cells) possess the ability to self-renew and give birth to normal cancer cells to grow the tumor.[46] Scientists are currently investigating the root source of CSCs. Although many different factors contribute to the rise of CSC in a tumor, the EMT has been suspected for its cause in giving cancer cells the capability of cancer stem cells.[47] Mesenchymal cells, a product of the EMT, can differentiate into multiple lineages.[48] More cancer-associated EMTs lead to an increased amount of migratory cells that can form new tissues and metastasize. CSCs, in squamous cell carcinoma and breast cancer, exhibit both epithelial and mesenchymal characteristics in a shifting manner.[49] They can be proliferative like epithelial cells and migratory like mesenchymal cells.[50] Cells, after experiencing the EMT, increase their tumor size by at least 10-fold, which further supports the notion that the EMT gives cells characteristic of stem cells.[51]

Twist thwarts the expression of CD24, which will eventually lead to cells with CSC's phenotypes.[52] Snail triggers the dedifferentiation of epithelial cells in colorectal cancer.[53] ZEB1, an EMT transcriptional factor, can restrict many epithelial determinants and consequently dedifferentiate cancer cells.[54] In gastric, breast, liver, and colon cancer, the existence of Snail influences the dedifferentiated phenotype of cancer cells, conveying that differentiated cancer cells transform into less differentiated cancer cells after undergoing EMT.[55] These less differentiated cancer cells exhibit a more CSC-like phenotype.

The EMT confers cancer cells not only stemness properties but also mesenchymal properties.[56] For instance, breast cancer cell lines with a high population of CD44+/CD24- cells exhibit both stem/progenitor cell properties and mesenchymal markers.[57] Similar findings were also observed in nontumorigenic immortalized human mammary epithelial cells (HMLEs).[58] Ectopic expression of Twist or Snail induces EMT, a process in which mesenchymal-like cells with a CD44 high/CD24 low pattern are created.[59] This unsurprisingly indicates that HMLEs become more stem-like as a result of undergoing the EMT. These acquired stem cell properties can also be demonstrated using functional assays.[60] Scientists had long been testing cancer cells' ability to form tumorspheres to determine their state of stemness. When Snail or Twist is upregulated, HMLEs form more than 30-fold the amount of its current tumorspheres.[61] Additionally, the number of CSCs increases two-fold as Twist or Snail, EMT-inducing transcription factors, are overexpressed.[61]

Researchers have already evaluated breast cancer cells' state by examining their CD24 expression.[62] They found that CD24-negative mesenchymal-like cells, unlike CD24-positive epithelial-like cells, were able to form tumorspheres and therefore expand their tumor.[62] Cells transitioned from CD24-positive to CD24-negative through EMT, which is induced by TGF-β.[63] EMT factor Snail confers the cancer cells the plasticity they need to migrate and have stem cell properties.[64] Many researchers conclude that cells undergoing EMT become dedifferentiated. Some researchers even used mice skin carcinoma cell lines as models to track the effect that activation of Snail can have on primary tumors.[65] They found that when Snail is activated through EMT, cells begin to have higher tumor-initiation capacity.[65] These observations give strong support to our understanding that the EMT gives stemness traits to cancer cells. As discussed before, EMT triggers a set of early steps that are pivotal to the development of cancer cell metastasis. After the EMT, cells have to revert to their original epithelial state so that they can colonize a new environment (a site different from where the original tumor grows) and establish a metastatic tumor.[66] Mouse breast cancer cells can turn mesenchymal through spontaneous EMT, which causes them to disperse.[67] But after this, they transform back into their epithelial state to settle their metastasized tumor.[67]

Mechanism of EMT-induced Cancer Stemness

How the EMT mechanism gives cancer cells acquired stemness traits is still not totally understood.[68] However scientists can approach this by examining the EMT transcription factors, such as Twist, Snail, and Zeb, which can modulate non-coding RNA expression.[69] miRNAs are small non-coding RNA molecules significantly affecting cell differentiation as they function in the post-transcriptional period of gene expression regulation.[70] Therefore, the factors that affect the expression and processing of miRNA could play a pivotal role in the cells' stemness or differentiation.

miRNAs that promote differentiation include the let-7 family and miR-34. The let-7 family can be observed in all somatic cells, so they can be seen as an obstacle to maintaining cells' stemness.[71] Scientists have classified the let-7 family as a group of tumor suppressors due to their pluripotency and various oncogenic traits.[71] For instance, let-7 can inhibit Lin28, a gene responsible for self-renewal within cells.[72] As a result, decreased expression of Myc and Sall4 occurs.[72] Scientists are still understanding the mechanism in which let-7 loss happens. Let-7 genes can be removed through the reprogramming of somatic cells using additional transcription factors such as Oct4, Sox2, Klf4, and c-Myc, which induce pluripotency into differentiated cells.[73]

In cellular reprogramming, the inhibition of let-7 allows for the pluripotency targets to be expressed, thus increasing efficiency.[74] When Snail binds to the let-7 promoters, its expression increases while that of let-7 decreases.[75] In pluripotent stem cells, low levels of let-7 are maintained by Lin28, which prevents the processing and production of let-7.[76] Additionally, Twist, another one of three main EMT transcription factors, also represses let-7 expression.[77] Thus, it can be concluded that the EMT factors are a major repression source of let-7 and other tumor-suppressor miRNAs.

Like let-7, miR-34 inhibits pluripotency factors so that cells don't acquire stemness traits.[78] As a p53 target, its expression is likely dysregulated in p53-mutated cancers.[79] When Snail represses miR-34 expression by binding to the miR-34 promoter, cells in EMT develop their stemness phenotype.[80]

Aside from let-7 and miR-34, many alternative miRNAs could influence the establishment of the stem cells in tumors. Despite the power let-7 has in differentiating cells, it lacks in comparison to the power of miRNAs that activate self-renewal, called ESCC (ESC-specific cell cycle) miRNAs.[81] The miR290 cluster is an example of ESCC miRNAs.[82] Therefore when let-7 is downregulated, cell self-renewal is more unlikely, especially in conjunction with the expression of ESCC miRNAs. This shows how the path that cells take to acquire stemness traits is complicated as there are "checks and balances" that keep the cell in its differentiated state.

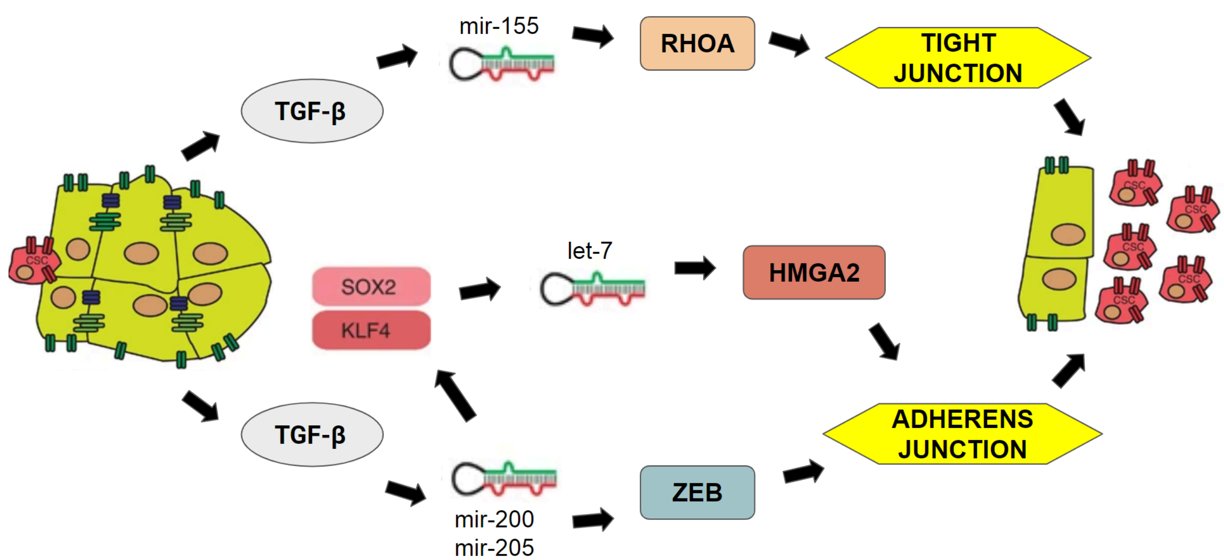

Figure 2: microRNAs can be seen as a mediator of EMT, which causes cancer cells' stemness. EMT induced by TGF-β is activated by mir-155, a microRNA that could target RHOA to break down tight junctions, which ultimately results in the creation of more cancer stem cells.[83] TGF-β can also modulate restrictive actions against mir-200 and mir-205, which increases levels of Zeb protein, which decreases levels of E-cadherin, which causes adherens junctions breakdown.[84] Increased levels of Zeb can also come back and repress mir-200 and mir-205 by promoting Sox2 and Klf4, two attackers of mir-200.[85] This triggers the renewal of cancer stem cells. TGF-β can also cause downregulation of E-cadherin by increasing HMGA2 activity.[86] The let-7 microRNA could block CSC's self-renewal and repress HMGA2, a molecule that helps in E-cadherin repression. Therefore, it can be inferred that there is a correlation between TGF-β signaling, EMT, and the emergence of CSCs.

Since 1991, the association between the loss of E-cadherin and the EMT causing tumor dedifferentiation has been observed. Recent discoveries show that the expression of individual EMT-inducing transcription factors such as Snail, Twist, or Zeb, can transform the cancer cells into cancer stem cells. ZEB1, an EMT transcription factor in carcinoma cells, is unusually overexpressed in pancreatic cancer, causing the stem-like phenotypes in previously non-stem-like cells.[87] ZEB1 has also been shown to be a significant player in the stage of tumor initiation in a xenograft model.[88] Similar to how Snail dedifferentiates cells by inducing expression of let-7 and miR-34, the EMT transcription factor ZEB1 inhibits many miRNAs such as miR-203, miR-200, and miR-183 so that stemness factors such as Sox2, KLF4, and BMI1, get upregulated.[89] Hence, ZEB1 can be linked to the EMT and cancer cell dedifferentiation (stemness). EMT in ovarian cancer development turns the epithelial cells into stem-like cells called mesenchymal cells. EMT factors inhibit pathways and proteins including NF-kB, tumor necrosis factor alpha, β-catenin, and p53 so that cells don't differentiate and stay in their pluripotent state.[90]

Another way EMT factors can give cells stemness traits is through the prevention of senescence.[91] Somatic cells' life span is limited and when differentiation ceases, their dividing and self-renewing ability also cease. However, both stem and cancer cells are able to overcome this limit, which allows them to proliferate at a nonrestrictive pace. The EMT transcriptional factors Snail and Twist could suppress tumor suppressors, say, cycle regulator p16, which enhances the cancer cell's ability to self-renew and grow.[92] Thus, it can be concluded that there are many different molecular mechanisms at play in the process of cells gaining EMT-driven stemness. The EMT correlation with stemness can also be seen at a clinical level. Researchers use gene expression profiling to design a system that could quantitatively score tumors on their EMT state.[93] This system can identify the distinct EMT states across different tumor types.[94] The reported gene expression, given by the system's molecular subtypes, shows that they closely represent the EMT status in ovarian cancer. The prognosis of ovarian cancer patients is defined by clinicopathological parameters, such as the extent of metastasis damage and cell resistance to chemotherapy.[95] The higher the EMT score, the worse the prognosis. A higher level of pluripotency and stemness also indicates a higher level of chemoresistance and more chance of metastasis. It can therefore be logically inferred that subtypes with higher EMT scores are associated with the dedifferentiated state. These findings sum up to the conclusion that cancer cells acquire potency or stemness after undergoing the EMT, which shows how the EMT can cause dedifferentiation in cancer cells.

EMT-induced Therapeutic Resistance

Mediators of EMT enhanced not only cellular motility but also cellular survival. In an environment of serum starvation and TNF-α treatment, the expression of Snail in Madin–Darby Canine Kidney (MDCK) cells strengthens its resistance.[96] This anti-apoptotic cellular reaction was correlated with Snail expression, through the activation of both the MAPK and the PI3K pathways.[97] Through inhibiting pro-apoptotic factors such as p53, DNA Fragmentation Factor 40, and BH3-Interacting Domain Death Agonist, Slug that was transfected into MCF7 breast cancer cells causes cells' resistance to programmed cell death (due to DNA damage).[98] After scientists found that EMT links to enhanced survival pathways and anti-apoptotic behaviors, they are interested in exploring how EMT causes cells' resistance to anti-neoplastic therapeutic strategies.

Recent studies have investigated the cancer cells' acquired chemotherapy resistance due to EMT upregulation causing molecular alterations in gastrointestinal malignancies. Pancreatic cancer cells' acquired gemcitabine resistance showed changed behavior and phenotypes that aligned with EMT.[99] For example, the resistant pancreatic cancer cells experienced upregulated vimentin, lack of E-cadherin expression, and β-catenin nuclear translocation.[100] Applying chronic oxaliplatin exposure to CRC cells can trigger resistant EMT-correlated phenotypic mutations including loss of polarity, spindle shape, and increased mobility.[101] The oxaliplatin-induced resistant cells, as predicted, showed decreased levels of E-cadherin in sync with increased levels of snail and vimentin, all hallmarks distinctive of EMT.

These studies demonstrate that cancer cells undergo EMT to adopt a new anti-apoptotic and pro-survival state when they are induced with stress from chemotherapy. 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.[102] Therefore, EMT pathways can also be seen as a direct mechanism or mediator of chemotherapy resistance.[103] For instance, Panc-1 cancer cells with transfection of Snail adopted unusual EMT traits that make them sensitive to chemotherapy treatment such as 5-fluorouracil and gemcitabine.[104] More recently, manually-programmed expression of Snail in colon cancer cells increases the CSC population and phenotype that explains its oxaliplatin resistance.[105] Specifically, snail-expressing HCT116 and HT29 cells, two types of colorectal cancer stem cells, demonstrated EMT-linked morphological, functional, and molecular characteristics such as having a 10-fold resistance to oxaliplatin.[105]

While anti-gastrointestinal-malignancy drugs have shown partial success in compromising vascular endothelial growth factor and epidermal growth factor receptors, a fully successful treatment is still far away due to roadblocks such as therapeutics resistance. More importantly, scientists have examined the influence of EMT in these molecule-targeting therapy's outcomes. It was discovered that there was erlotinib resistance in head and neck squamous cell carcinoma.[106] Erlotinib is a tyrosine kinase inhibitor of epidermal growth factor receptors.[107] An upregulation of vimentin contrasting to the downregulation of claudin-4, E-cadherin, and claudin-7 that supported this resistance is what microassay and western blot analysis shows.[108] Conclusively, this change in protein expression pattern resembles distinguishable traits of cells undergoing EMT.

To further understand how EMT relates to drug resistance, researchers have used the tumor from patients, whose cancer cells prove the erlotinib to be ineffective, for investigation. What they found was that after being treated with erlotinib, tumors with E-cadherin depletion take a shorter time to progress compared to tumors with E-cadherin bulges. Unsurprisingly, non-small lung cancer cells that were treated with gefitinib (another tyrosine kinase inhibitor of epidermal growth factor receptor) also show the same pattern of low E-cadherin results in short time to progression and high E-cadherin results in long time to progression.[109] Levels of E-cadherin were measured by immunohistochemical staining.[110] Applying vascular endothelial growth factor receptor 1 and bevacizumab, an angiogenesis inhibitor targeting vascular endothelial growth factors, strengthen the multiple colon cancer cell lines' migratoratory and invasive properties.[111] These recent discoveries demonstrating EMT's pivotal role in acquired chemoresistance show that it is necessary for the development of novel drugs or therapies that could successfully inhibit EMT pathways, which would predictably improve patient outcomes when used in sync with traditional therapies such as chemotherapy and radiation.

Conclusion

During cancer progression, the synergization between EMT and CSCs contributes a major part to the survival, aggressiveness, and mobility of a tumor. EMT and CSCs can be seen as mediators of cancer development since they guard cancer cells against harmful environments or drugs and promote metastasis for long-term survival and worsened malignancy. Even though scientists are currently in a period where neoadjuvant treatment is commonly used, it should be carefully considered before use because these treatments could trigger more aggressive cancer phenotypes by conferring cancer cells the capability of metastasis and resistance. This concern suggests that researchers need to spend more time to further investigate the impact clinical therapy has on the pathobiological advancement of cancer. As discussed previously, methods blocking EMT pathways and subsequently canceling the maintenance of CSC prove to be future endeavors worthy of attention and trials. Luckily, this path is feasible as there have already been studies conducted to identify certain pharmacological agents capable of regulating the tumor's state of differentiation. CSCs, cancer cells in the dedifferentiated or pluripotent state, can be compromised through the promotion of their differentiating ability.[47]

Therefore, agents that could induce cancer stem cells to differentiate into a more niche, less pluripotent state, namely salinomycin or HDAC inhibitors, may hold significant therapeutic potential.[112] Alternatively, both the TGF-β and Wnt pathways could be targeted as a strategy to decelerate the rate of EMT, which will lead to downregulation of CSCs.[113] These pathways activate not only EMT but also anti-apoptotic signaling, including the ones involving PI3K and nuclear factor-κB. Since PI3K and Akt play a major role in EMT, inhibiting them with the purpose of eliminating EMT and the rise of CSCs may show promising results. Lastly, since microRNAs contribute to the modulation of EMT and CSC emergence, injections of microRNAs manually designed to decrease EMT can also be seen as another bright path towards disrupting cancer progression.

To sum up, researchers are having a much better understanding of how EMT carries out functions to improve cellular mobility and survival, which ultimately aids in tumor malignancy. It is well understood that EMT plays a pivotal role in the development of aggressive CSCs, no matter which organ the tumor originates from. Aside from being an effective marker of cancer aggressiveness and therapeutic resistance, EMT and CSC molecular pathways can also be targeted therapeutically to advance the fight against cancer.

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