

Epithelial-to-Mesenchymal Transition as a Potential Target for Antineoplastic Therapies

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Abstract

Epithelial-to-mesenchymal transition (EMT) is a multi-step reprogramming process resulting in a phenotype switch from an epithelial to a mesenchymal state. This phenotype switching of cells, long been studied for its role in development, is now emerging as a crucial process that endows tumor cells with migratory and invasive properties, enriches stem cell-like attributes, enhances drug resistance, prevents apoptosis and contribute to immunosuppression. A comprehensive understanding of EMT cellular program will enable identification and development of potential EMT-targeted antitumor therapeutic strategies. This Editorial briefly describes recent evidence of EMT as a driver of malignancy and evaluates various strategies to target EMT in cancer.

Keywords: EMT; Epithelial-to-mesenchymal transition; Cellular plasticity; Cancer stem cells; Drug resistance; Metastasis; Proteomics

Introduction

Metastasis and therapeutic resistance represents substantial obstacles to achieving favourable clinical response in cancer patients [1]. Phenotype switching from epithelial to mesenchymal cellular state is well recognised as a fundamental step in metastasis and in the acquisition of resistance to conventional antitumor therapy regimens [2]. It is essential to develop novel strategies focused at preventing therapeutic resistance and metastatic spread of malignant tumors by targeting key regulators of phenotype switching process.

Phenotype switching or epithelial-to-mesenchymal transition (EMT) is a complex molecular and cellular program by which epithelial cells lose their differentiated characteristics, including cell-cell adhesion, planar and apical-basal polarity and acquire mesenchymal features, including motility, invasiveness, ability to escape immune cells and a heightened resistance to apoptosis [3]. EMT is a crucial event that occurs during embryonic development to enable embryonic epithelial cells to transit into a mesenchymal state that is more amenable to cell movement and travel to distant sites where new tissues and organs form [4,5]. Conversely, mesenchymal cells can undergo a reverse phenotype switching to regain epithelial state via mesenchymal-to-epithelial transition (MET) [4,5]. In cancer the inappropriate induction of these developmental processes can be disastrous as they lead to the development of cancer cells that are drug

resistant, with stem cell-like properties and are able to invade locally and metastasize [5,6].

Clinical relevance of EMT in cancer

Previous studies that assessed EMT markers in clinical tumor samples have yielded mixed results and lead to scepticism regarding its relevance in cancer. Although some studies showed lack of conclusive evidence for EMT in clinical tumors specimen, other studies provide compelling evidence of EMT in promoting tumor progression [7,8]. The potential reasons for this discrepancy may be attributed to the tiny fraction of cancer cells that undergo an EMT and technical difficulties of capturing this transient and reversible process [9]. A growing body of evidence now indicates that EMT plays a central role during tumor invasion and metastatic dissemination [5,9,2]. In various cancers, a link between EMT and generation of cancer cells with stem cell attributes of tumor initiation and resistance to chemotherapy have been established [10,2]. EMT has been implicated with acquisition of drug resistance in clinical tumor samples. For instance lung cancer cells in the mesenchymal state exhibit greater resistance to epithelial growth factor (EGFR) kinase inhibition treatment than the epithelial state [11]. Circulating tumor cells (CTCs) obtained from peripheral blood of breast cancer patients frequently show evidence of EMT [12]. Furthermore, significant correlations between EMT and patient prognosis have been demonstrated in multiple cancers. For instance EMT is associated with advanced clinical stage and poor prognosis in prostate cancer [13].

The diverse molecular mechanisms that contribute to EMT including transcription factors and multiple signal pathways have been reviewed by Lamouille et al.,

[4]. On a molecular level, EMT is frequently assessed by a cadherin switch with the loss of E-cadherin by epithelial cells and concomitant upregulation of N-cadherin by mesenchymal cells [14]. In addition to these two cellular states, intermediate EMT states displaying simultaneous existence of both epithelial and mesenchymal characteristic have been recently observed in many cancers [15]. These intermediate or hybrid EMT phenotypes reflect enhanced plasticity of tumors and are important for understanding the progression of EMT/MET process. Taken together, these data suggest that inhibition of epithelial-mesenchymal plasticity is an attractive approach for therapeutic intervention aimed at inhibiting cell-state transitions.

Strategies to effectively target EMT in cancer therapy

Given the role of EMT in enhancing tumor cell invasion, drug resistance and stemness, developing EMT-targeted therapies could potentially serve to decrease metastasis, overcome drug resistance and impact the stem cell component of tumors. Efforts have been made to develop antineoplastic therapies that directly target EMT [16]. However, most of the data on drugs targeting EMT has to be regarded as preliminary and further research is warranted to demonstrate its clinical benefits in cancer patients. Strategies proposed to target EMT to develop novel cancer therapeutics include identification of novel druggable EMT components by high throughput drug screen and proteomics approaches and specifically targeting EMT components by utilising appropriate animal models and immunotherapeutic approaches.

Identification of druggable EMT components by high throughput approaches

The rapidly evolving field of proteomics mass spectrometry offers several avenues to understand the molecular basis of EMT at various levels (translational, post-translational, interaction networks, signalling pathways, etc). Broadly, these strategies can be classified into either 1) global or 2) targeted proteomic analysis. A global profiling with an integrated systems biology approach can be used as an initial assessment to identify all proteins present in a specific condition or cell type. The reference proteome library thus generated would form the basis for any differential quantitative analysis between various conditions. Numerous protein and peptide labelling techniques like iTRAQ and TMT-plex isobaric labelling, Stable Isotope Labelling by Amino acids in Cell culture (SILAC), isotopic peptide dimethylation, etc among others, can be used in conjunction with tandem mass spectrometry to identify and quantify 1000s of proteins between various samples in a single experiment [17,18,19,20]. A comparison between epithelial, mesenchymal and hybrid cell proteomes, for example, would provide useful insights into differentially regulated proteins and their altered signalling pathways. In addition, mass spectrometry also allows for the identification and quantitation of post-translational modifications (phosphorylation, glycosylation, etc) that would prove to be vital for the EMT process. Specific enrichment strategies can be applied to isolate post-translationally modified molecules and analyse their role in EMT. Novel EMT components thus identified using a global proteomics approach, can further be validated using targeted proteomic strategies. The arrival of powerful, label-free and highly sensitive technologies like Multiple Reaction Monitoring (MRM), SWATHMS (ABSciex), Intact protein profiling (Bruker Corporation), etc among others, provides high resolution quantitative protein data from complex samples to confidently validate EMT markers. Integration of EMT proteomics data with existing gene expression data would therefore be the right path to advance in our understanding of EMT.

High-throughput drug screening approach to identify compounds that specifically target epithelial, mesenchymal or hybrid states may lead to the development of specific novel therapies. One such approach resulted in the identification of Salinomycin that demonstrated significant toxicity against breast cancer cells with mesenchymal and stem cell attributes [21]. However, the exact mechanism of action of Salinomycin remains to be elucidated to estimate its applicability in clinics.

Targeting EMT with appropriate animal models and immunotherapeutic approaches

Due to the transient and rare nature of the EMT process, there is a paucity of animal models for studying motile behaviour of cancer cells in vivo. There are ongoing efforts to replicate the physiological events occurring during metastatic dissemination of tumors in animal model systems [22]. As the molecular mechanisms for tissue interaction, penetration and remodelling seen during EMT in cancer appear to have much in common with embryonic EMT, developmental animal models are being adapted for cancer studies. For example, the embryonic chicken transplantation model has emerged as a useful system to assess melanoma cells invasion and EMT. This model exploits the ancestral relationship between melanoma and its precursor neural crest cells [23]. We have used this model to target candidate EMT genes in vivo by perturbing gene expression with siRNA approach [14,24]. Incorporating developmental animal models in cancer studies should prove valuable for validation of molecules involved in metastatic behaviour as well as for the development of therapies that target related pathways.

Cancer cells that have undergone EMT acquire immunosuppressive properties to escape immune-mediated destruction. A recent study showed that EMT signature from multiple cancer types was associated with enrichment of multiple druggable immune targets [25]. This has potentially important implications for identifying cancer patients who may benefit from immunotherapies. Additionally, cancer vaccines-based approach in which a specific T-cell immune response can be elicited against an important EMT component appears to be a viable option for eradicating cells undergoing an EMT. For instance the tumor-restricted pattern of expression of the transcription factor Brachyury and its proven immunogenicity made it an appealing protein for a cancer vaccine strategy targeting EMT. Brachyury-specific CD8+ cytotoxic T cells capable of killing Brachyury-expressing tumor cells in an HLA-restricted manner were recently developed [9]. Cancer vaccines constitute a very attractive methodology as they are specific, generate long lasting antitumor effects and have low toxicities.

Conclusion

In summary, targeting of EMT represents a potentially novel approach for preventing metastasis, overcoming drug resistance and stemness. There is the need to characterize the distinct cellular states associated with epithelial-mesenchymal plasticity in order to identify better druggable EMT components and design treatment strategies to effectively eliminate cells undergoing EMT. Better preclinical models are required to validate the effective targeting of EMT components. This will be crucial to understand how to clinically prevent metastasis and drug resistance and to give mechanistic insights to be translated into therapeutic opportunities. However, significant additional work is needed to translate these findings into meaningful therapies. It remains to be seen whether single inhibitors of EMT or combination chemotherapy might acquire synergistic effects and improve treatment response.

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