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Mini Review

The Role of Epithelial-Mesenchymal Transition in the Development of Resistance in Non-Small Cell Lung Cancer

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Abstract

Non-Small Cell Lung Cancer (NSCLC) has poor prognosis and remains difficult to treat mainly due to the development of therapeutic resistance. Epithelial-Mesenchymal Transition (EMT), a process that results in epithelial cells acquiring mesenchymal cell properties, is believed to play an integral role in cancer by promoting cancer progression and resistance to molecularly-targeted therapies. EMT regulation is dependent on a complex interplay of numerous key mediators and signaling pathways, which could be the cause of resistance to several recently developed NSCLC molecularly-targeted therapies. However, acquired resistance due to alternative signaling pathways poses a major concern for the efficacy of these molecularly-targeted therapies, particularly with their long-term use; hence, combinatorial therapies have been proposed to circumvent acquired resistance. Further investigation into EMT biomarkers may be beneficial for addressing acquired resistance in NSCLC patients.

Keywords: Epithelial-mesenchymal transition; Tyrosine kinase inhibitor; Drug resistance; Lung cancer; Non-small cell lung cancer

Abbreviations

NSCLC: Non-Small Cell Lung Cancer; EMT: Epithelial-Mesenchymal Transition; TCF4: T-CellEnhancer 4; mTOR: Mammalian Target of Rapamycin; TGF-β: Transforming Growth Factor -β; EGFR: Epidermal Growth Factor Receptor; RTK: Receptor Tyrosine Kinase; MAPK: Mitogen Activated Protein Kinase; TKI: Tyrosine Kinase Inhibitor; VEGFR: Endothelial Growth Factor Receptor; ALK: Anaplastic Lymphoma Kinase; HER2: Human Epidermal Growth Factor Receptor 2; PRMT1: Protein Arginine N-Methyltransferase 1

Introduction

Despite advances in diagnosis and treatment, lung cancer remains the leading cause of cancer-related mortality in the United States. Non-Small Cell Lung Cancer (NSCLC) accounts for the majority of all lung cancer cases that are diagnosed, with a 5-year survival of 49% and 45% for patients diagnosed at stage IA and IB respectively. While the most advanced stage IV has a 5-year survival rate of about 1%, according to the American Cancer Society Facts and Figures 2016. Unfortunately, most lung cancer patients, at time of diagnosis, are diagnosed at an advanced stage of the disease, where the efficacy of chemotherapy is low. Lung cancer is classified into subtypes on the basis of histopathalogy and gene expression of various biomarkers [1,2]. Lung tumors are cellularly and molecularly heterogeneous with subsets of lung cancer cell populations exhibiting distinct molecular and phenotypic features [3]. Therefore individualized treatment based on tumor subtypes is not always effective as treatment of cancers with multiple cell clones results in elimination of the sensitive clones and expansion of resistance clones giving rise to therapeutic resistance [4]. Acquired therapeutic resistance poses a major challenge to the success of treatment, and several mechanisms of resistance have been identified. Studies indicate that EMT, a transdifferentiation process typically observed during embryonic development and wound healing, plays a crucial role in cancer progression and phenotypic changes contribute to tumor heterogeneity due to acquisition of mesenchymal cell characteristics resulting in development of resistance to many molecularly-targeted NSCLC therapies. Currently, further studies are required to target EMT biomarkers to reverse the process of EMT and prevent cancer progression.

What is EMT

Epithelial-Mesenchymal Transition (EMT) is a physiological process in which epithelial cells lose cell polarity and cell-cell adhesive properties while acquiring motile and invasive properties of mesenchymal cells. Acquisition of a mesenchymal phenotype gives cells the ability to disseminate to distant sites through the blood or lymphatic vessels. Activation of EMT in NSCLC cells promotes tumor metastasis due to the attainment of invasive characteristics and cytoskeleton remodeling, resulting in drug resistance and tumor progression [5].

Numerous key players have a role in the regulation of EMT, making this a complex, integrated, and well-coordinated process. An important initial step in EMT is the down regulation of E-cadherin, which is responsible for epithelial cell-cell adhesion and maintenance of the cytoskeleton organization. On the other hand, up-regulation of N-cadherin results in NSCLC metastasis and inhibition of N-cadherin has been shown to decrease NSCLC proliferation and invasion [6]. E-cadherin repression is accomplished through several transcriptional factors including ZEB1, ZEB2, SNAIL1, SNAIL2, Twist1, Twist2, and E12/E47 [7]. Due to their role in the induction of translation suppression and mRNA degradation, micro-RNAs

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also play a role in EMT regulation [8]. Studies have shown several post-transcriptional regulatory miRNAs to be involved, such as miR-200, miR-101, and miR-506 The mammalian Target Of Rapamycin pathway (mTOR) has also been identified as a regulator of EMT, motility, and metastasis of colorectal cancer, although its role in lung cancer has not been found [9].

The induction of EMT can also occur through growth factors such as Transforming Growth Factor-β (TGF-β), which activates a complex signaling cascade producing a transcriptional response and promoting EMT.Production of TGF-β is increased in cancer cells, thus promoting EMT as well as angiogenesis, which provides a route for the mobile mesenchymal cells to access distant metastatic sites [10]. Recently it was identified that miR-145 and miR-203 inhibited TGF- β -induced EMT and invasion through repression of SMAD3 in NSCLC cells [11]. Reports have also suggested that that attenuated LKB1-SIK1 signaling can promote EMT and increase the radioresistance of NSCLC cells, thereby enhancing their overall metastatic potential [12]. This suppression of EMT by LKB1-SIK1signaling could be through the repression of ZEB1, a potent driver of EMT and hence targeting the LKB1-SIK1-ZEB1 pathway to suppress EMT might provide therapeutic benefits to NSCLC patients [13]. Another recent study revealed that Mammalian Eps15 Homology Domain 1 (EHD1) induces EMT and promotes metastatic behavior of cells in NSCLC [12]. Additionally, inflammatory cytokines and hypoxia typically observed in the tumor microenvironment have been proven to promote EMT [14]. EMT can also occur due to the acquisition of cancer stem cell-like characteristic of mesenchymal origin, which may contribute to drug resistance. Reports have demonstrated that cells undergoing EMT can acquire stem cell-like properties by expressing stem cell markers BMI1, ALDH1A1 and CD133 [15]. However, the molecular mechanism through which tumors acquire cancer stem cell-like properties and drug resistance remain unclear.

EMT & drug resistance

EMT can also be mediated by the over expression and amplification of Receptor Tyrosine Kinases (RTKs), such as EGFR and c-Met receptors, and by their subsequent activation by the appropriate ligands EGF and HGF, respectively. It has been shown in NSCLC cells that both EGFR and c-Met are overexpressed or activated and c-Met can often be amplified [9,16]. Over expression and amplification of these RTKs can have multiple profound effects on many downstream signaling pathways. For instance, when mutated, these RTKs can lead to uncontrolled amplification and up regulation of numerous downstream signaling pathways such as Mitogen-Activated Protein Kinase (MAPK), Phosphoinositide 3-Kinase (PI3K)/AKT, and mammalian Target Of Rapamycin (mTOR) pathways, which are responsible for the regulation of angiogenesis, cell proliferation, migration, and cell death or survival [17]. Due to the profound effects of RTK overexpression/amplification, many molecularly targeted NSCLC therapies that target RTKs have been developed in an effort to reverse the effects of EMT. One such class is a group of small molecule inhibitors that inhibit the tyrosine kinase domain of EGFR and are thus referred to as Tyrosine Kinase Inhibitors (TKIs). The first TKIs shown to have clinical efficacy was erlotinib [18]. Targeting EGFR in the patients with small molecule inhibitors showed success in the clinic in patients who had a mutation in the EGFR TK domain. However, the vast majority of these patients developed resistance within 9-14 months to the EGFR targeted molecular treatment [19]. It has been shown that TKI resistant NSCLC cell lines which were made resistant to TKI after treating with erlotinib for prolonged time, underwent EMT [19]. Hence in NSCLC cell lines, occurrence of EMT is initially induced by prolonged treatment with TKIs and once cells underwent EMT, cells showed no response to TKIs because of their mesenchymal characteristic. Other targets of these therapies include: Endothelial Growth Factor Receptor (VEGFR), Anaplastic Lymphoma Kinase (ALK), and Human Epidermal Growth Factor Receptor 2 (HER2). The role of EMT in inducing resistance to TKIs is currently poorly understood and needs further studies.

Unfortunately, a major problem with molecularly-targeted therapy is the development of acquired drug resistance through phenomena like EMT, which results in a reduced overall treatment efficacy [20]. This phenomenon has been previously explored and it has been found that acquired resistance may be the result of activation of alternative signaling pathways that are involved in the EMT process [21-23]. It has been shown previously by our lab that in Tyrosine Kinase Inhibitor (TKI)-resistant cells, alternative signaling pathways, such as Wnt and mTOR, may lead to the induction of EMT [20]. When EGFR and c-Met are upregulated, they can signal downstream to mTOR and MAP kinase pathways, thus activating the Wnt/β-catenin pathway. Our studies also indicate that when both EGFR and c-Met are inhibited by their respective TKIs, the production of Wnt7b is upregulated through the activation of the Wnt/β-catenin pathway by the formation of a feedback loop that continuously stimulates the mTOR and MAP kinase pathways. Hence, the usage of combinatorial therapies that can inhibit Wnt and mTOR pathways, along with EGFR and c-Met inhibition, could overcome acquired TKI resistance.

Another EMT biomarker that may play a role in TKI resistance is Protein Arginine N-Methyltransferase 1 (PRMT1), an enzyme that catalyzes the methylation of arginine in proteins [24]. PRMT1 has been shown to be overexpressed in the early stage NSCLC [25]; furthermore, studies have shown that PRMT1 is a novel EMT regulator/inducer, as PRMT1 catalyzes the methylation of arginine in Twist1 resulting in repression of active E-cadherin [24]. Previously proposed mechanisms of PRMT1-induced EMT demonstrate that PRMT1 forms a complex with Src-1, which is stimulated by RAS, causing an E-cadherin to N-cadherin switch. It has also been shown in a previous study that gene silencing of PRMT-1 leads to the reversal of EMT in NSCLC cells, hence PRMT-1 may be a novel EMT regulator [24]. Nuclear accumulation of β-catenin induces the expression of ZEB1, an EMT biomarker [26], which downregulates E-cadherin in cancer cells and induces tumor progression [27]. MiR-200a, a translational repressor of ZEB1, is downregulated during EMT through the upregulation of β-catenin [28], thus allowing ZEB1 to repress E-cadherin and EMT to proceed. It has been shown in SU11274 and erlotinib-resistant H2170 cells that expression of E-cadherin was restored when miR-200a mimics were introduced and TKI-resistant cells had increased sensitivity to TKIs [19]. However, more studies are required to fully understand the role of miR-200a in inducing EMT in TKI resistance; furthermore, utilization of miR-200a may also be useful in the development of more effective therapies for EMT in TKI-resistant NSCLC cases.

Since activation and crosstalk by several signaling pathways can mediate EMT, it may be overcome by combinatorial therapies,

which are a relatively new development for the treatment of NSCLC. A recent phase I clinical trial investigated using a combination of celecoxib, an inhibitor of Cyclooxygenase-2 (COX-2) and erlotinib, an inhibitor of EGFR, in NSCLC patients and showed promising results. COX-2 is able to mediate resistance to EGFR TKIs via PGE2-dependent promotion of EMT, which works through the subsequent downregulation of E-cadherin by upregulation of ZEB1 and Snail [29]. Consequentially, PGE2 is able to activate the MAPK/ERK signaling pathway, which leads to the development of EMT. In addition, overexpression of Metalloproteinases (MMPs), which are associated with tumor invasion, may lead to further tumor progression [30]. This study demonstrated a correlation between matrix metalloproteinase-9 levels and tumor response, when treated with a combination of celecoxib and erlotinib [31]. Thus, this study demonstrated that treatment with a combination of COX-2 inhibitors and EGFR inhibitors could modulate several markers of EMT that are associated with patient tumor responses. Recently it was reported that overexpression of Cadherin-2 (CDH2), promoted invasion and induced EMT in erlotinib-resistant lung cancer cell lines [6]and MiR-124 could suppress the expression of CDH2 and regulate EMT in NSCLC cells [32]. In another study, Metformin, a treatment for diabetes that has anticancerous properties, was used in tandem with gefitinib or erlotinib in both EGFR-TKI-resistant lung cancer cells and a mouse xenograft model. The study showed that when cells were treated with Metformin and gefitinib or erlotinib, expression of Snail and Vimentin (a mesenchymal phenotype marker) were downregulated and E-cadherin were upregulated and the cell morphology changed from a mesenchymal spindle-like phenotype to an epithelial phenotype [33]. The study also concluded that Metformin, when used either alone or in combination with erlotinib or gefitinib, can reduce the size of tumors in mice xenografts.Co-treatment with a dual PI3K and mTOR inhibitor, BEZ235 and HDAC inhibitor Trichostatin A ,TSA can synergistically inhibit NSCLC cell proliferation and also suppress NSCLC migration, invasion and the Epithelial-Mesenchymal-Transition [34]. Based on the aforementioned studies and crosstalk between signaling pathways causing EMT and EGFR activation, the development of other combinatorial treatments may reverse EMT and improve patient prognosis.

Conclusion

Several molecular-targeted therapies for NSCLC modulate EMT signaling pathways. EMT is a complex process involving crosstalk between multiple signaling pathways and transcriptional factors to accomplish transition of epithelial cells to a mesenchymal phenotype. Unfortunately, long-term treatments typically lead to the acquisition of resistance to therapy, possibly due to the activation of alternative signaling pathways. By studying the mechanism of therapeutic resistance and activation of alternative pathways mediating EMT and investigating key EMT biomarkers, new strategies to overcome drug resistance and metastasis may be discovered and current therapies can be improved.

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