Inside this issue: Epithelial-mesenchymal transition (EMT) in lung disease

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Dear APSR Colleagues:

Epithelial-to-mesenchymal transition (EMT) is a developmental program that involves drastic phenotypic changes, including transformation to motile and invasive phenotypes. During the last decade, studies have revealed the critical roles of EMT in carcinogenesis. By undergoing EMT, cancer cells acquire enhanced metastatic potential, drug resistance, and even stemness characteristics. Initial advances in this field were made mostly in breast and colorectal cancers. However, a number of recent studies have revealed the critical roles of EMT in lung cancer, including important new findings reported in the last year. For example, Sequist et al. demonstrated the occurrence of EMT in patients who developed epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor resistance. In addition, investigators, including ourselves, have identified the predominant role of ZEB1, a master EMT-inducing transcription factor, in EMT in lung cancers. Further research is required to translate these findings to the clinic, as EMT-targeted therapy obviously has the potential to dramatically improve the situation for patients with lung cancer.

Sincerely,

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**Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors**

Authors: Sequist LV et al.


URL: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3132801/?tool=pubmed](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3132801/?tool=pubmed)

Comment: Lung cancers with active mutant epidermal growth factor receptors (EGFRs) frequently respond to EGFR-tyrosine kinase inhibitors (TKIs), but inevitably acquire resistance. Previous studies identified two major molecular mechanisms responsible for resistance to TKIs, including the T790M point mutation and MET amplification. This is a very important paper, and the first to demonstrate, by serial biopsies, the occurrence of EMT, which is associated with EGFR-TKI resistance, in patients with EGFR mutant lung cancer. More strikingly, this study showed that transformation to small cell lung cancer occurs in a subset of patients who develop acquired EGFR-TKI resistance.

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**Epithelial to mesenchymal transition in an epidermal growth factor receptor-mutant lung cancer cell line with acquired resistance to erlotinib**

Authors: Suda K et al.


Comment: This paper also provides data from an *in vitro* cell line model, suggesting that EMT is a potential mechanism for acquired EGFR-TKI resistance. The authors developed a resistant sub-line (HCC4006ER5) harbouring an *EGFR* mutation, from the non-small cell lung cancer (NSCLC) HCC4006 cell line, by chronic exposure to increasing concentrations of erlotinib. They analyzed the mechanism of resistance to the TKI and found that HCC4006ER5 cells did not exhibit the known TKI-resistance mechanisms. A genome-wide gene expression analysis revealed that HCC4006ER5 cells exhibited a change in gene expression indicating EMT. This study suggests that EMT may be an important mechanism for acquired resistance to EGFR-TKIs in *EGFR* mutant NSCLC.
Slug confers resistance to the epidermal growth factor receptor tyrosine kinase inhibitor

Authors: Chang TH et al.


URL: http://ajrccm.atsjournals.org/content/183/8/1071.long

Comment: This study demonstrated an active role for Slug, a key regulator of EMT, in the development of resistance to the EGFR-TKIs. The investigators clearly showed that upregulation of Slug was associated with gefitinib resistance, and that silencing of Slug restored the gefitinib sensitivity of PC9 cells harbouring an EGFR mutation. They also showed that Slug enhanced tumour formation in a xenograft mouse model. Furthermore, analysis of clinical samples demonstrated the consistent finding that Slug expression was significantly higher in cancer cells that were resistant to EGFR TKIs than in cancer cells that were responsive.

L1CAM protein expression is associated with poor prognosis in non-small cell lung cancer

Authors: Tischler V et al.


URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3198986/?tool=pubmed

Comment: Growing evidence has highlighted the importance of EMT in carcinogenesis, in cell lines and animal models, but to date there have been few studies demonstrating the clinical relevance of EMT. This study showed that expression of the L1 cell adhesion molecule (L1CAM) was associated with the mesenchymal phenotype and poor prognosis in patients with NSCLC. Importantly, the study showed that membrane E-cadherin was expressed at the centre of the tumour, whereas L1CAM and Slug were expressed at the invasive front. This is the first study to demonstrate EMT through the tumour environment in pathological sections of tumours from lung cancer patients.
Epithelial to mesenchymal transition is a determinant of sensitivity to chemoradiotherapy in non-small cell lung cancer

Authors: Shintani Y et al.
URL: http://www.sciencedirect.com/science/article/pii/S0003497511017929
Comment: The standard treatment for locally advanced non-small cell lung cancer (NSCLC) is combined modality therapy with both chemotherapy and radiotherapy. Although phase III studies have shown the clinical effectiveness of concurrent chemoradiotherapy for stage III NSCLC, the 5-year survival rate was approximately 20%. This is likely to be due to the resistance of NSCLC to these therapies. Recent studies have demonstrated the contribution of EMT to resistance to chemotherapy with cytotoxic drugs. The investigators tested the hypothesis that EMT may result in resistance of NSCLC to chemoradiotherapy. They assessed the correlation between EMT and sensitivity to chemotherapy or radiotherapy, using NSCLC cell lines and tumour specimens from patients with NSCLC, before and after chemoradiotherapy. Chronic exposure to cytotoxic agents or radiation caused A549 NSCLC cells to undergo EMT. The EMT status of NSCLC specimens obtained after chemoradiotherapy was correlated with shortened disease free survival. Furthermore, multivariate analysis revealed that EMT status was an independent variable predicting disease free survival. This study indicates that EMT is an important mechanism for resistance to chemoradiotherapy, not only in vitro, but also in clinical settings, suggesting that novel therapeutic strategies targeting EMT signalling are needed, to overcome the resistance of NSCLC to chemoradiotherapy.

Epithelial to mesenchymal transition by TGFβ-1 induction increases stemness characteristics in primary non small cell lung cancer cell line

Authors: Pirozzi G et al.
URL: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0021548
Comment: The cancer stem cells (CSC) hypothesis has attracted great interest in the biomedical community because it suggests the possibility that therapies targeted at CSC may eradicate tumour cells. A recent study by the Weinberg group demonstrated a link between EMT and CSCs. The investigators demonstrated a relationship between EMT and CSCs by using a LC31 lung cancer primary cell line. LC31 cells underwent EMT in the presence of 2 ng/mL TGFβ-1, and this was characterized by a morphological change to a fibroblast-like appearance, up-regulation of mesenchymal markers, and down-regulation of epithelial markers. The authors demonstrated up-regulation of stem markers such as Oct4, Nanog, Sox2, c-kit and CD133, as well as enhanced pneumosphere formation in EMT-induced LC31 cells. Finally, they showed increased tumorigenicity of EMT-induced LC31 cells in immunosuppressed mice. They concluded that the induction of EMT by TGFβ-1 treatment in a lung cancer cell line resulted in the acquisition of a mesenchymal profile that was associated with up-regulation of stem cell markers.
**Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation**

Authors: Chiou SH et al.

Reference: Cancer Res 2010; 70: 10433-44.

URL: [http://cancerres.aacrjournals.org/content/70/24/10433.long](http://cancerres.aacrjournals.org/content/70/24/10433.long)

**Comment:** The transcription factors, Oct4 and Nanog, have been suggested to be one of four defined factors capable of reprogramming adult cells into germline-competent induced pluripotent stem (iPS) cells. This paper reported the critical roles of Oct4 and Nanog in the epithelial to mesenchymal (EMT) and cancer stem cell (CSC)-like maintenance of lung cancer. The authors found that co-expression of Oct4 and Nanog was frequent in lung adenocarcinoma (LAC) samples. Next, they showed that exogenous expression of Oct4 and Nanog in LACs enhanced their stemness features. Simultaneous knockdown of Oct4 and Nanog reversed the EMT process through suppression of the expression of Slug, an EMT-inducing transcription factor. Finally, they showed that triple positivity for Oct4/Nanog/Slug correlated with a worse prognosis in LAC patients. The authors concluded that Oct4/Nanog signalling contributes to both EMT and CSC-like properties in lung adenocarcinomas.

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**Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells**

Authors: Takeyama Y et al.


URL: [http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3110825/?tool=pubmed](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3110825/?tool=pubmed)

**Comment:** Developmental genetics studies have resulted in the discovery of several master EMT-inducing transcription factors, including ZEB1, SIP1 (also known as ZEB2), Snail, Slug, and Twist. These transcription factors are capable of inducing EMT when ectopically expressed in epithelial cells. In this study we show that among four master EMT-inducing genes, ZEB1 expression was most significantly correlated with a mesenchymal phenotype in NSCLC cell lines and tumour tissues. Importantly, silencing of ZEB1 in NSCLC cell lines dramatically suppressed their anchorage-independent growth, which is one of the hallmarks of cancer cells. These results underscore the pivotal role of ZEB1 in EMT in lung cancer.
ZEB1-responsive genes in non-small cell lung cancer

Authors: Gemmill RM et al.
Reference: Cancer Lett 2011; 300: 66-78.
URL: http://www.sciencedirect.com/science/article/pii/S0304383510004301

Comment: This study delineates the essential role of the ZEB1 gene as a regulator of EMT in lung cancer. Using genome-wide gene expression analysis, the authors identified sets of genes, the expression of which was positively or negatively correlated with ZEB1 expression, in 38 NSCLC cell lines. By performing over-expression or knockdown experiments for ZEB1 they confirmed that eight genes selected from these sets were regulated by ZEB1. A mesenchymal gene expression pattern was associated with ZEB1 and ZEB2, but not with other EMT-inducing transcription factors, which is consistent with the data from our study, as described previously.

A comparison of Twist and E-cadherin protein expression in primary non-small-cell lung carcinoma and corresponding metastases

Authors: Wang G et al.
URL: http://ejcts.oxfordjournals.org/content/39/6/1028.full

Comment: The investigators demonstrated that expression of Twist, a master EMT-inducing transcription factor, was significantly higher in metastatic non-small cell lung carcinoma tissues than in primary non-small cell lung carcinoma tissues, and that E-cadherin expression was higher in the primary tumour than in metastatic lesions. They also showed that increased Twist expression was correlated with decreased membrane expression of E-cadherin. Therefore, they concluded that Twist induces EMT in non-small cell lung carcinoma by repressing E-cadherin, thereby contributing to metastases.

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