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Epithelial–Mesenchymal Transition and Immune Evasion during Lung Cancer Progression: The Chicken or the Egg?

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Summary

Epithelial–mesenchymal transition (EMT) is a complex process involved in metastasis. Immune evasion is required for tumor progression and is characterized by an ineffective anti-tumor immune response and upregulation of immune suppressive signals. The co-existence of EMT and adaptive immune evasion opens the possibility of a mechanistic link between these processes.

In this issue of *Clinical Cancer Research*, Lou and colleagues (1) report an epithelial to mesenchymal transition (EMT)-related mRNA signature associated with increased expression of diverse immune inhibitory ligands and receptors in lung adenocarcinomas (e.g. PD-L1/2, PD-1, TIM-3, LAG-3, B7-H3, BTLA and CTLA-4). In addition, tumors harboring the EMT-signature displayed higher levels of Th1-inflammation markers than the epithelial-like malignancies (e.g. IFN- γ and CXCL-10); and enrichment of CD4+/FoxP3+ immune suppressive regulatory T-cells (Tregs). Notably and despite their prominent biological differences, tumors in both EMT-categories showed a comparable amount of non-synonymous mutations. Taken together, these results indicate a previously unrecognized connection between EMT-mediated tumor progression and activation of an immune escape program in lung tumors. Moreover, the results point to a possible role of EMT markers as candidates for prediction of response to immune checkpoint blockade in lung adenocarcinomas.

Consistent with the findings by Lou and colleagues (1), a recent study found a similar association between EMT and immune suppressive responses in cultured breast carcinoma cells (2). In human breast tumors, the presence of an mRNA-based EMT signature or increased Vimentin/reduced E-cadherin protein was significantly correlated with PD-L1 upregulation (particularly in claudin-low triple negative breast carcinomas). Down-regulation of PD-L1 using shRNA strategies reduced the mesenchymal phenotype in breast cancer cells, suggesting a bi-directional cross-talk between EMT and PD-L1 mediated

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immune evasion (2). Additional studies will be required to determine the association between EMT and immune escape mechanisms in other tumor types.

During EMT, the epithelial tumor cells undergo transformation from a cohesive, polar phenotype to a motile-mesenchymal state lacking the characteristic adherence through epithelial cell-cell junctions. EMT is a complex, heterogeneous process involving genetic and epigenetic modifications as well as profound alterations of the tumor microenvironment (3). Since EMT is considered to be a crucial step in the metastatic cascade, it has been an area of active research in diverse epithelial malignancies (4). However, clear identification and measurement of EMT is limited by the lack of reliable biomarkers and the complex/dynamic nature of the process. Moreover, EMT might not be a defined tumor state, but rather a continuum representing cellular plasticity where some cells have more epithelial and some more mesenchymal characteristics, whose extremes look like EMT phenotypes.

Immune evasion is a key aspect of malignant tumor progression and is characterized by either the absence of detectable anti-tumor immune reaction (e.g. so-called “*immune ignorance*”) or by the progression of the tumor in the presence of an ineffective anti-tumor immune response (4). The biological determinants of the different pathways used by tumor cells to evade immunity are not completely understood but are likely related with the balance between tumor antigenic load and the presence of adaptive immune suppressive mechanisms. The balance between tumor inflammation and immune evasion is considered to be a dynamic process and solid tumors can display immune heterogeneity with the same lesion containing areas with dissimilar immune infiltration.

The co-existence of features of EMT and adaptive immune response/evasion in lung adenocarcinomas opens the possibility of a mechanistic link between these processes. In addition, this association suggests that tumor progression through EMT is accompanied by increasing anti-tumor immune pressure. Adaptation of the tumor through upregulation of immune inhibitory signals and expansion of regulatory cells could support tumor progression under this condition (**Figure 1**). In this regard, key EMT-related transcription factors, members of the Snail and Zeb families, have been directly linked with immune suppressive effects in cancer (3). For instance, Snail1-induced EMT was associated increased CD4⁺/FoxP3⁺ regulatory T cells and impaired dendritic cell function in melanoma models (5). In addition, the Zeb1/miR-200 axis was shown to modulate the levels of PD-L1 in lung cancer cells even in the absence of IFN- γ , suggesting that mesenchymal-like tumor cells are intrinsically capable of immune escape (6). Another mechanism possibly linking EMT and immune suppression is aberrant β -catenin signaling. During the destabilization of adherens junctions in EMT, E-cadherin is cleaved and degraded, altering the β -catenin intracellular location and signaling. In human melanomas and animal models, active tumor cell β -catenin signaling was associated with profound reduction of T-cell infiltration and limited response to immune checkpoint blockers (7).

Alternatively, inflammatory signals can also induce EMT in cancer cells. Treatment of lung cancer cells with TGF- β induces a Smad4/miR-124 mediated EMT phenotype (8). Exposure of carcinoma cells to TNF- α , IL-6, IL-10 and IL-8 can directly induce EMT-related transcription factors and favor a mesenchymal phenotype in tumor cells (3). In addition,

immune suppressive cells such as alternatively activated/polarized macrophages (so-called “M2-type macrophages”) have also been shown to induce EMT when co-cultured with pancreatic carcinoma cells through IL-10 mediated signaling (9). Similarly, the contact between myeloid derived suppressor cells and nasopharyngeal carcinoma cells resulted in COX-2/TGF- β mediated EMT (10). Possible common inducers of EMT and immune suppression include sustained (e.g. chronic) inflammation, hypoxia and metabolic depletion in the tumor microenvironment.

Identification of highly sensitive/specific predictive biomarkers for PD-1 axis therapies is a major unmet need in the field of immuno-oncology. To date, biomarkers associated with increased response to PD-1 targeting antibodies in solid tumors include PD-L1 protein expression, tumor inflammation/inflammation-related signatures, increased T-cell receptor clonality and elevated mutation/class-I neoantigen load (11-14). To date, the performance of these tests as single markers is suboptimal and most have not been standardized for clinical use. However, refinement of the available biomarkers and establishment of predictive multi-parametric signatures is a matter of active research.

It is tempting to speculate that stratification of lung adenocarcinoma patients based on EMT-phenotype could aid in selection of patients that are more likely to benefit from immune checkpoint blockade. However, the prominent association between EMT-features, tumor immune infiltration, INF- γ -related signals and PD-L1 expression in lung tumors, suggests overlap with the available biomarkers. Future studies will be required to identify *bona fide* and relatively simple EMT markers to test their predictive role in lung adenocarcinoma as well as in other tumor types.

In summary, the presence of EMT-like features can be associated with inflammation and upregulation of immune suppressive signals/targets in human carcinomas. The mechanistic determinants and directionality of this association are not well understood, but seem to be complex and self-perpetuating. Although preliminary observations suggest a possible role of EMT-markers as predictors of response to PD-1 axis therapies in lung adenocarcinomas, future work will be required to reliably measure EMT in tumor specimens and determine its predictive value in patients treated with immunostimulatory therapies. Evaluation of the association between EMT and immune activation/suppression in additional lung cancer subtypes such as squamous cell carcinomas and neuroendocrine small-cell tumors is also warranted.

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Figure 1. Possible association between EMT and anti-tumor immune response during (epithelial) cancer progression

Although EMT and tumor immune rejection/evasion are continuous, heterogeneous and potentially reversible processes, they frequently co-exist in lung adenocarcinomas suggesting a mechanistic link between them. Key EMT-mediators such as Snail, Twist, Zeb and aberrant β -catenin signaling can induce immune suppressive features in tumors. However, diverse inflammatory mediators such as TGF- β , TNF- α , IL-6, IL-8 and IL-10 or immune suppressive cells can promote an EMT-program in carcinoma cells. APC, antigen-presenting cell.