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CCR Translations

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

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Epithelial–mesenchymal transition (EMT) is a complex process involved in metastasis. Immune evasion is required for tumor progression and is characterized by an ineffective antitumor immune response and upregulation of immune-suppressive signals. The coexistence of EMT and adaptive immune evasion opens the possibility of a mechanistic link between these processes. *Clin Cancer Res; 22(14); 3422–4. ©2016 AACR.*

See related article by Lou et al., p. 3630

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., IFNγ and CXCL-10); and enrichment of $CD4^+/FoxP3^+$ immune-suppressive regulatory T cells (Tregs). Notably and despite their prominent biologic 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). Downregulation of PD-L1 using short hairpin RNA strategies reduced the mesenchymal phenotype in breast cancer cells, suggesting a bidirectional cross-talk between EMT and PD-L1–mediated 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). As 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 antitumor immune reaction (e.g., so-called "immune ignorance") or by the progression of the tumor in the presence of an ineffective antitumor immune response (4). The biologic 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 immunosuppressive 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 coexistence 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 antitumor immune pressure. Adaptation of the tumor through upregulation of immune inhibitory signals and expansion of regulatory cells could support tumor progression under this condition (Fig. 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

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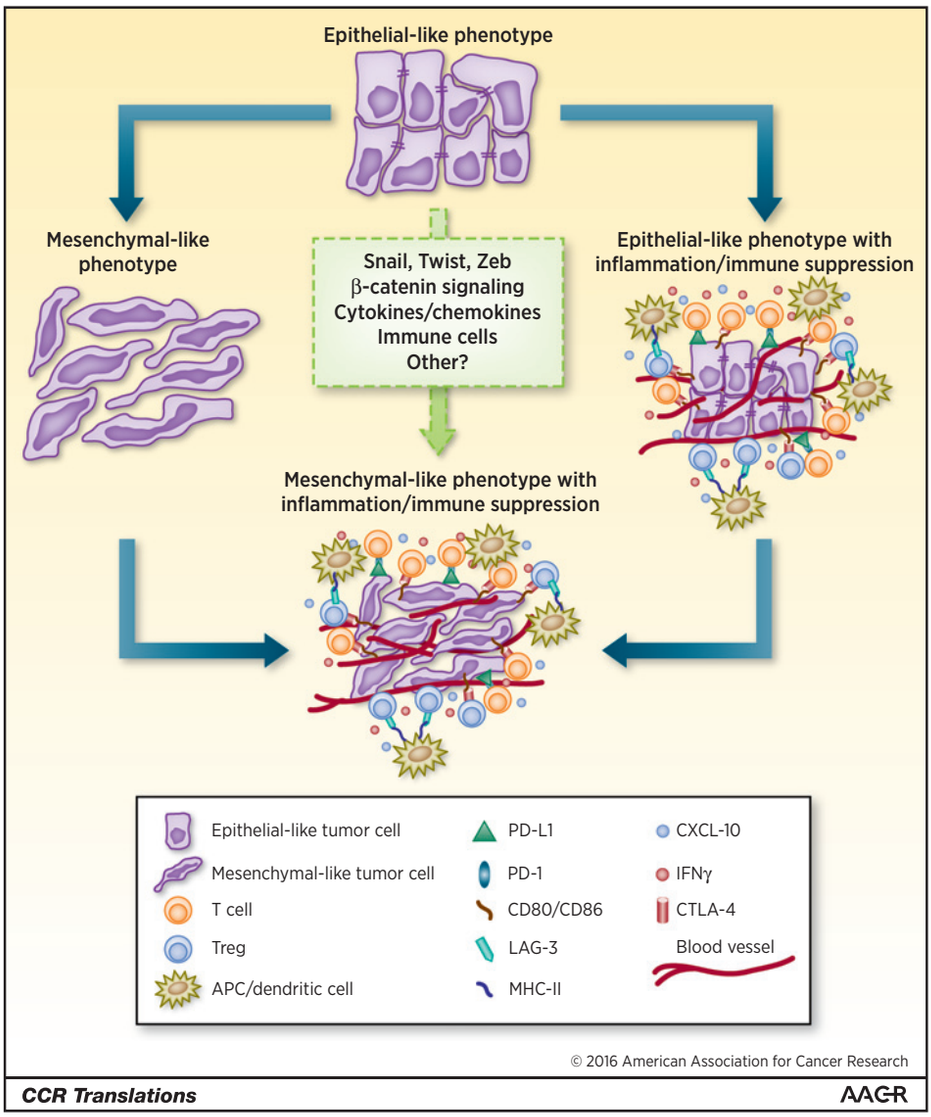

Figure 1.
Possible association between EMT and antitumor immune response during (epithelial) cancer progression. Although EMT and tumor immune rejection/evasion are continuous, heterogeneous, and potentially reversible processes, they frequently coexist in lung adenocarcinomas suggesting a mechanistic 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 TGFβ, TNFα, IL6, IL8, and IL10 or immune-suppressive cells can promote an EMT program in carcinoma cells. APC, antigen-presenting cell.

associated with increased $CD4^+/FoxP3^+$ Tregs 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 IFNγ, 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 TGFβ induces a Smad4/miR-124–mediated EMT phenotype (8). Exposure of carcinoma cells to TNFα, IL6, IL10, and IL8 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 cocultured with pancreatic carcinoma cells through IL10-mediated signaling (9). Similarly, the contact between myeloid-derived suppressor cells and nasopharyngeal carcinoma cells resulted in COX-2/TGFβ-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). The performance of these tests as single markers is suboptimal and most have not been

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standardized for clinical use. However, refinement of the available biomarkers and establishment of predictive multiparametric 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, IFNγ-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.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: I. Datar, K.A. Schalper
Development of methodology: K.A. Schalper
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.A. Schalper
Writing, review, and/or revision of the manuscript: I. Datar, K.A. Schalper
Study supervision: K.A. Schalper
Other (figure design): I. Datar

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References

1. Lou Y, Diao L, Cuentas ERP, Denning WL, Chen L, Fan YH, et al. Epithelial–mesenchymal transition is associated with a distinct tumor microenvironment including elevation of inflammatory signals and multiple immune checkpoints in lung adenocarcinoma. Clin Cancer Res 2016;22:3630–42.
2. Alsuliman A, Colak D, Al-Harazi O, Fitwi H, Tulbah A, Al-Tweigeri T, et al. Bidirectional crosstalk between PD-L1 expression and epithelial to mesenchymal transition: significance in claudin-low breast cancer cells. Mol Cancer 2015;14:149.
3. Pietilä M, Ivaska J, Mani SA. Whom to blame for metastasis, the epithelial-mesenchymal transition or the tumor microenvironment? Cancer Lett 2016 Jan 11. [Epub ahead of print].
4. Zheng H, Kang Y. Multilayer control of the EMT master regulators. Oncogene 2014;33:1755–63.
5. Kudo-Saito C, Shirako H, Takeuchi T, Kawakami Y. Cancer metastasis is accelerated through immunosuppression during Snail-induced EMT of cancer cells. Cancer Cell 2009;15:195–206.
6. Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn YH, Byers LA, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nat Commun 2014;5:5241.
7. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic beta-catenin signalling prevents anti-tumour immunity. Nature 2015;523:231–5.
8. Zu L, Xue Y, Wang J, Fu Y, Wang X, Xiao G, et al. The feedback loop between miR-124 and TGF-beta pathway plays a significant role on non-small cell lung cancer metastasis. Carcinogenesis 2016;37:333–43.
9. Liu CY, Xu JY, Shi XY, Huang W, Ruan TY, Xie P, et al. M2-polarized tumor-associated macrophages promoted epithelial-mesenchymal transition in pancreatic cancer cells, partially through TLR4/IL-10 signaling pathway. Lab Invest 2013;93:844–54.
10. Li ZL, Ye SB, OuYang LY, Zhang H, Chen YS, He J, et al. COX-2 promotes metastasis in nasopharyngeal carcinoma by mediating interactions between cancer cells and myeloid-derived suppressor cells. Oncoimmunology 2015;4:e1044712.
11. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 2014;515:563–7.
12. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014;515:568–71.
13. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015;372:2018–28.
14. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science 2015;348:124–8.

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