Comment on Neoantigen-directed immune escape in lung cancer evolution

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Abstract

Understanding how a tumor evolves and avoids immune recognition is paramount to improving cancer immunotherapy and patient outcome. Here we examine our recent integration of multi-region genomic, transcriptomic, epigenomic, pathology, and clinical data, highlight the need for a systematic examination of immune escape mechanisms, and discuss implications for immunotherapy approaches.

Main text

Cancer is an evolutionary process, reliant on genetic diversity and subject to selective forces, including those from the immune microenvironment. By recognizing antigenic components of the tumor cell, the immune system may exert an evolutionary pressure, shaping the antigenicity of the tumor as it evolves. Subclonal populations of tumor cells, either lacking immunogenic antigens or able to withstand an immune response, may be selected for (1,2).

By analyzing genomic, transcriptomic, epigenomic, pathology, and clinical data, it is possible to decipher a tumor's evolutionary history and the corresponding shifts in the immune contexture. This will allow a better understanding of the complex interplay between an evolving cancer and a dynamic immune microenvironment. In our recently published work, we performed an integrated analysis of 258 regions from 88 early-stage, untreated NSCLC from the 'Tracking Non-Small-Cell Lung Cancer Evolution through Therapy' (TRACERx) clinical study (3). Using this cohort and its

associated multi-region genomic, transcriptomic, epigenomic, pathology, and clinical data, we determined how levels of immune infiltration varied between and within tumors and subsequently characterized mechanisms acquired by the tumor that may have aided immune evasion and their associations with clinical outcome.

By clustering tumor regions based on their immune infiltration, we could classify tumors as being uniformly “immune cold”, uniformly “immune hot”, or heterogeneously infiltrated (if the same tumor had both “immune cold” and “immune” hot regions). Strikingly, we found that nearly a third of patients had tumors with disparate levels of immune infiltration between regions, highlighting a potentially confounding factor for the use of transcriptomic biomarkers in predicting response to immune-checkpoint blockade. This suggests a single tumour sample is often not representative of either the genomic landscape nor the immune microenvironment of the tumour (4).

Through previous work by our group and others, many immune evasion mechanisms have already been identified including: HLA loss or down-regulation of expression (5-7), B2M loss and other antigen presentation defects (8), and the elimination of neoantigens through copy number loss (9). We found the majority of tumours harboured at least one mechanism of immune evasion, highlighting the strong selection pressure which the immune microenvironment imposes on a growing tumour. We also identified a novel epigenetic mechanism of immune evasion through neoantigen expression down-regulation via promoter hypermethylation of genes that contain neoantigenic mutations.

Interestingly, we found that the mechanisms of neoantigen presentation dysfunction observed in a tumor differed according to its immune infiltration landscape. “Immune cold” tumors were enriched for copy-number loss of previously clonal neoantigens and showed a decrease in immunoediting during tumor evolution; “Immune hot” tumor regions exhibited either HLA LOH or depletion of expressed neoantigens. This suggests an ongoing process of immune-editing in highly infiltrated tumors and historical, or no longer active, immune-editing in sparsely infiltrated tumors.

Finally we developed the “immune-evasion capacity” metric to combine our assessment of the immune infiltration landscape with the identified immune escape mechanisms in each tumor. Tumors with a low immune-evasion capacity were those that had high immune infiltration or no identified mechanism of immune escape. After determining this score for every patient’s tumor,

we observed that it could forecast disease-free survival – patients whose tumors had a low immune-evasion capacity exhibited significantly longer disease-free survival.

Taken together, our results highlight the dynamic nature of cancer evolution and the ongoing interplay between the cancer cell and the immune microenvironment. While our results provide only a single snapshot of the genomic, transcriptomic and epigenetic landscape of the cancer cell, they suggest the observed phenotype represents a product of continuous immune sculpting. These results may have important implications for existing immunotherapy and neoantigen vaccine approaches.

The observation of historic loss of neoantigens through copy number events (analogous to a fossil record) in immune cold regions raises the tantalising possibility that these tumour regions once were replete with immune cells. Conceivably, such immune-cold regions could therefore be induced to become immune-hot again, possibly through targeting neoantigens still present in the region, or through modulating signaling molecules responsible for recruiting immunosuppressive cell types (10,11).

Likewise, the observation that neoantigens may be silenced through promoter methylation suggests that these neoantigens could be re-activated through, for example, demethylating agents. Such an approach may also result in increased expression of MHC. Ongoing clinical trials are currently evaluating the effect of combining epigenetic modifiers with immune therapies (12). The transient nature of transcriptional repression and epigenetic silencing could also help explain why some tumors respond a second time following disease progression to checkpoint inhibitor blockade (13) and highlight the importance of longitudinal and dynamic profiling, as currently only a single point in time of a tumor's immune/epigenomic landscape is considered.

A deeper understanding of the immune microenvironment and how it can change over time, both in the context of treatment-naive tumours and those treated with immunotherapy is needed. These results also have implications for the design of neoantigen vaccines. In addition to the complexities already surrounding target neoantigen selection, such as mutation class, clonality, binding affinity, diversity of HLA presentation, antigen processing, and similarity to self, it will also be imperative to carefully evaluate the expression of putative neoantigen targets, and moreover, consider the likelihood of copy number or expression loss being selected as the tumour

evolves. Further, they highlight the importance of integrating genomic and transcriptomic data to elucidate prime immune targets.

Finally, our study underscores the need to systematically examine different mechanisms of immune escape. Other mechanisms of expression down-regulation, beyond promoter hypermethylation, certainly exist, which may be identified in larger cohorts, such as the expanded TRACERx cohort. Additional projects like RUBICON (a rule book and immune atlas for combination therapy) that aim to map out the immunological landscape and infiltrating cell types of TRACERx tumors in exhaustive detail, will be instrumental in understanding precisely what makes a tumor region hot or cold and the complexity of the tumor/immune microenvironment.

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R.R., N.M., and C.S.jointly conceived of and wrote the manuscript.

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stock options in and has consulted for Achilles Therapeutics and holds a European patent in determining HLA LOH (PCT/GB2018/052004).

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