ID: 00913
Type: Oral Comunication
Topic: Tumor microenvironment

Characterization of several immune-related genes in oncogenic-driven lung tumors by direct mRNA digital counting

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Introduction

Programmed death ligand 1 (PD-L1) expression is constitutive activated in oncogenic-driven nonsmall cell lung cancer (NSCLC) leading to an anergic tumor microenvironment. However, the correlation between other immune-related genes and major NSCLC driver mutations in NSCLC remains poorly characterized. At this regard, gene expression profiling incorporating not only PD-L1 but also other components of the stroma might better capture the immunecontexture of these oncogenic subgroups of patients. NanoString's nCounter platform performs direct mRNA measurement without any enzymatic reaction allowing a more accurate evaluation of low-abundance or degraded mRNA obtained from formalin-fixed paraffin-embedded (FFPE) samples.

Objectives

To understand the immunogenic features of NSCLC, we simultaneously characterized seven key genes regulators of immune-response comprising CD4, CD8, programmed cell death-1 (PD-1), programmed death-ligand 1 (PD-L1), interferon gamma (IFNG), granzyme M (GZMM) and forkhead box P3 (FOXP3) and evaluate their significance among most relevant oncogenic-driven NSCLC tumors by using an alternative expression method based on direct mRNA digital counting (NanoString's nCounter).

Methodology

Sequence-specific probes for the 7 immune-related genes (CD4, CD8, PD-1, PD-L1, IFNG, GZMM, FOXP3) were designed and included in a customized nCounter panel (NanoString Technologies) used in our institution on a routine basis to simultaneously screen for most relevant oncogenicdriver genes (ALK, ROS1, RET, NTRK1 and METex14). A total of 296 advanced NSCLC patients from two different institutions were analyzed with nCounter and driver mutations were interrogated with next-generation sequencing (NGS, Ion Torrent PGM or Gene-Reader). Total mRNA (nCounter) and DNA (Ion Torrent) required for analysis were obtained from FFPE tissue samples. Among them, 115 patients (38.9%) representative of the larger oncogenic subgroups were used for final intercomparison (KRAS mutant [n=33], ALK rearranged [n=44] and wild-type (WT) [n=38]). Analyses of variance (ANOVA) were used to describe statistical differences in the expression of the selected immune-related genes among the molecular-selected subgroups.

Results

In our series of advanced NSCLC patients, oncogenic drivers (ALK, KRAS) were mutually exclusive. The analysis of the 7-gene 'signature' revealed distinct expression patterns among the molecular-selected subgroups for evaluation. A significantly higher mRNA expression of CD4 and PD-L1 ($p=0.0014$ and $p=0.0467$, respectively) was observed in ALK rearranged tumors compared to the KRAS mutant and WT groups. No significant differences were observed for the other immune-related genes (CD8, PD-1, IFNG, FOXP3, GZMM). A significant linear correlation between the expression of CD4 and PD-L1 was noted in ALK positive patients ($p= 0.0214$), but not in KRAS mutant samples ($p= 0. 112$) suggesting a strong positive association between both genes in ALK-rearranged tumors. Unsupervised hierchical clustering analysis of individual immune genes across all samples (n=296), identified sets of correlated genes: PD-1 and FOXP3 ($r=0.9$) and PD-1 with GZMM ($r=0.8$).

Conclusions

ALK-rearranged NSCLC tumors show a distinctive immune-contexture when compared to KRAS mutated and WT tumors. Our data reinforce previous observations of an increased PD-L1 expression in ALK-driven tumors. However, we could not observe any significant increase in other genes related to an immunosuppressive microenvironment such as FOXP3 regulatory T cells. We are currently working on expanding the sample size in order to gain further information in other molecularly selected subsets of NSCLC tumors.

Acknowledgements

This work has been granted by the Spanish Ministry of Health, Instituto de Salud Carlos III (FIS16/00890) and Pfizer.

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