Editorial

Prognostic value of biomarkers in the tumor microenvironment of pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is the deadliest solid malignancy and the most common form of pancreatic cancer. It is the fourth prominent cause of worldwide cancer-related deaths with a 5-year overall survival of less than 8% (1). Despite improved knowledge about its genetic characterization, PDAC remains insusceptible to most of the currently available treatment procedures, while complete surgical resection is the only viable option for the cure (2). Novel immunotherapies have shown encouraging results across multiple solid tumors; unfortunately, immunotherapies in PDAC have been disappointing (3). This unresponsiveness may, in part, be attributed to PDAC's acquired immunosuppressive tumor microenvironment (TME), driven by poor T cell infiltration, a low tumor mutational burden (TMB), and dense fibrotic stroma (3,4).

Better clinical outcomes can be achieved through molecular profiling and accurate subtyping upon detection and using customized therapeutic strategies. Molecular subtyping of cancers can be accomplished by unsupervised clustering of molecular data or hypothesis-driven classification based on biological and clinicopathological parameters. In the past, several PDAC molecular subtyping systems were proposed based on genomic variation, transcriptomics, epigenomics, stroma status, immunological status, and proteomics data (5-11). In recent years, the role of TME and its composition have gained much interest in PDAC molecular subtyping and therapeutics.

In *Annals of Translational Medicine*, Pu *et al.* analyzed 179 PDAC patients' clinical, gene expression, and somatic mutation data from The Cancer Genome Atlas (TCGA) (12). They estimated both the immune and stromal scores of each patient by using the Estimation of STromal and Immune cells in MAlignant Tumours using Expression data (ESTIMATE) tool (13) based on the immune and stromal signature gene expression levels. These scores were used to stratify PDAC patients into high- and low-score groups. Further, a Tumor Immune Estimation Resource (TIMER) tool (14) was used to assess infiltration levels of the $CD8^+$ T cells (cytotoxic), $CD4^+$ T cells (helper), B cells, Macrophages, Neutrophils, and dendritic cells (DCs). The patient cohort with higher immune and stromal scores have a higher level of infiltration of all of these immune cells except for $CD4^+$ T cells, while the cohort only with higher stromal score has lower $CD4^+$ T cell infiltration.

The study suggests that ductal adenocarcinoma group patients have higher immune and stromal score compared to other types of pancreatic cancers. There is no statistically significant difference in the overall survival and recurrence-free survival between high and low scoring groups of patients. Further, they also looked at the stromal and immune score of PDAC patients based on mutations in four highly mutated genes (*KRAS*, *TP53*, *SMAD4*, and *CDKN2A*). *KRAS* mutant group has significantly low immune and stromal scores compared to wildtype. *TP53* wildtype has significantly low immune score compared to wildtype, but there is no significant difference in the case of the stromal score. However, there is no significant difference in the stromal and immune scores of the

mutant groups of *SMAD4* and *CDK2A* compared to their corresponding wildtypes.

To explore the role of altered genes in PDAC, the authors analyzed differentially expressed genes (DEGs) in the high stromal and immune score groups against corresponding low score groups using Bioconductor tool *limma*. They observed an overlap of about 30% of the DEGs between the immune and stromal group analyses. Gene ontology (GO) and pathway enrichment analyses show the enrichment of immune response and cancer-related biological processes. To explore the role of DEGs on patients' survival, they performed a log-rank test and observed that several DEGs are associated with both the overall and recurrence-free survival of the PDAC patients.

Our own study on the PDAC patient data from TCGA suggests that several of these genes, e.g., *CCL2*, *CD226*, *CLEC17A*, *CNR2*, *CSF3R*, *CTSG*, *DPEP2*, *KLHL6*, *MAL*, *PLA2G2A*, *RASGRP2*, *RELN*, and *SCARA5* are associated with patients' overall survival (15). Functional enrichment analysis showed that these genes are also involved in adaptive immune response, chemokine-mediated signaling, and inflammatory response, etc. Tumor-promoting and pro-survival roles of inflammatory chemokine, C-C chemokine ligand 2 (CCL2) in pancreatic cancer was established earlier (16), but its role in TME has not been explored. In this study, Pu *et al.* report the pro-survival role of CCL2 in the PDAC TME. These results are corroborated by recent reports on the pro-survival role of CCL2 in the TME of breast cancer (17) and lung cancer (18). While our previous report suggests that CCL2 is underexpressed in the PDAC (15), by taking into consideration of ours and Pu *et al.* studies, we can deduce that tumor tissue cell downregulates CCL2 in TME to make it more aggressive.

In the current study, *AMH* (anti-müllerian hormone) and *TNNT1* (troponin T1) genes are associated with a better prognosis of PDAC, which is partly in line with our observation on the role of TNNT1 in the better prognosis of PDAC patients; however, there was no significant difference in the expression level of TNNT1 in PDAC patients compared to the normal (15). On the other hand, we didn't find any association between AMH and survival in PDAC patients in our study (15), even though Pu *et al.* report finds AMH's expression is associated with survival in PDAC patients with high stromal score. A recent report also suggests the pro-survival role of the *AMH* gene expression in lung cancer (19). CD226 (Cluster of Differentiation 226) encodes a co-stimulatory glycoprotein, DNAX accessory molecule-1 (DNAM-1) on the surface of T cells, natural killer (NK) cells, monocytes, and B cells. Overexpression of CD226 is associated with T and NK-cell mediated cytotoxicity against tumor cells (20), which regulates immune response in TME along with the T cell co-inhibitory receptor, TIGIT [T cell immunoreceptor with immunoglobulin and tyrosine-based inhibitory motif (ITIM) domain] (21). In the current analysis, they observed that the expression of CD226 has a pro-survival role in PDAC TME, which is on expected lines (20).

Protein-protein interaction network analysis of survival-associated DEGs (82 in high-immune score group and 58 in high-stromal score group) with the STRING database identified highly interconnected *CNR2* and *CCL22* genes, which overexpressed in T cells, B cells, dendritic cells, NK cells, and macrophages (22,23). CCL22 is a well-known chemokine that recruits Treg (regulatory T cells) to suppress the immune response in the tumor tissue, and many types of human tumors are known to express high levels of CCL22 (24). In pancreatic cancer, CCL22 is produced by dendritic cells in TME, while cancer cells themselves do not secrete CCL22 *in vitro* or *in vivo* (24). The higher expression level of CCL22 is associated with immunosuppression in TME; hence, we can expect that it would lead to immune escape and poor prognosis. In contrast, the current report showed that high CCL22 expression was associated with increased overall survival in PDAC; a similar trend was also observed in breast cancer (25). Overall, it's convincible that the ratio of stroma and immune cell and altered expression of immune and stroma associated genes in TME is associated both with the overall and recurrence-free survival of the PDAC patients.

In conclusion, Pu *et al.* analyzed the altered expression of genes that are associated with TME composition in PDAC. Functional analysis of these DEGs suggests their involvement in immune-related pathways and TME. This study provides a list of genes with potential prognostic value to PDAC patients due to their association with the overall and recursion-free survival of PDAC patients. The mechanistic role of these marker genes is yet to be established fully with further experimental studies. In the future, we need to apply data from other publicly available large cohorts of patients to establish the role of genes associated with higher immune and stromal scores in *in vivo*, *in vitro*, and PDAC patient samples.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/atm.2020.03.59). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the works in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

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