Editorial

Yes-associated protein and immunosuppressive microenvironment in pancreatic cancer development: a new strategy to improve immunotherapy efficacy?

Rossana Berardi, Alessandro Bittoni

Clinica Oncologica, Università Politecnica delle Marche, Ospedali Riuniti di Ancona, Ancona, Italy

Correspondence to: Prof. Rossana Berardi. Clinica Oncologica, Università Politecnica delle Marche, Ospedali Riuniti di Ancona, Via Conca 71, 60126 Ancona, Italy. Email: r.berardi@univpm.it.

Provenance: This is an invited Editorial commissioned by Section Editor Dr. Chunlin Ou (Cancer Research Institute of Central South University, Changsha, China).

Comment on: Murakami S, Shahbazian D, Surana R, *et al*. Yes-associated protein mediates immune reprogramming in pancreatic ductal adenocarcinoma. Oncogene 2017;36:1232-44.

Submitted Jun 08, 2017. Accepted for publication Jun 10, 2017.
doi: 10.21037/jtd.2017.06.60
View this article at: http://dx.doi.org/10.21037/jtd.2017.06.60

Despite extensive research efforts and recent advances achieved in chemotherapy treatment of metastatic disease, pancreatic ductal adenocarcinoma (PDAC) prognosis remains dismal and it has been estimated that PDAC deaths will be second only to lung cancer by 2020 in United States (1). Therefore new therapeutic strategies are urgently needed.

Immunotherapy is a novel treatment approach that has been investigated over the last few years in a variety of cancers with encouraging results. Recent studies have demonstrated that development and progression of PDAC are influenced by immune response and inflammation pathways, suggesting that immunotherapy may represent a promising strategy. A peculiar feature of PDAC is represented by its microenvironment characterized by marked desmoplasia and a cellular infiltrate predominantly composed of fibroblast, leukocytes and endothelial cells. Despite the presence of immune cells at variable levels, PDAC is characterized by immune disfunction and tumor cells escape from the host immunosurveillance.

Pancreatic tumor cells have developed several mechanisms to modulate the immune system and avoid detection by effector cells, such as secretion of soluble immunosuppressive factors, including galactin-1 or transforming growth factor beta (TGF-β) (2) or downregulation of major histocompatibility complex (MHC) class I expression. Immune checkpoint modulation is one of the mechaninsms by which tumor cells control local immune response. Programmed cell death protein 1 (PD-1) is a coinhibitory receptor that downregulates T-cell activity in peripheral tissues during inflammation, preventing collateral tissue damage and development of autoimmunity. PD-1 is activated by two ligands, PD-L1 and PD-L2 both upregulated during an inflammatory response. PDAC has been shown to upregulate PD-L1 as a mechanism to restrain T-cell response decreasing cytokine production and T-cell proliferation (3). Moreover, PDAC are able to attract and activate immune cell populations with regulatory functions such as CD4+ CD25+ FOXP3+ regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). In particular, MDSCs are immature myeloid cells able to directly suppress T cells functions by depleting amino acids critical for T cell activation, producing nitric oxide and reacting oxygen species that suppress T cell signaling and downregulating selectins required for T cell homing to lymph nodes (4). Moreover, MDSCs inhibit T cell activity by secreting suppressive cytokines, as interleukin-6 (IL-6) and IL-10 or transforming growth factor-β (TGF-β) and preventing activation of T cells by antigen-presenting cells (APDAC). Interestingly, high concentration of MDSCs in the peripheral blood was associated with poor outcomes in patients with pancreatic cancer (5).

In the paper by Murakami *et al*. published on *Oncogene* in March 2017 (6), Yes-associated protein (Yap) has been demonstrated to be a critical regulator of

immunosuppressive microenvironment in PDAC, both *in vitro* and *in vivo*. Yap is a transcriptional coactivator of the TEAD family of transcription factors involved in regulation of the expression of a several anti-apoptotic and pro-proliferative genes. In PDAC mouse models, YAP has been shown to be an essential promoter of mutant KRAS oncogenic program, specifically inducing the expression of secreted factors as CTGF and CYR61 (7) and regulating the expression of Epithelial to Mesenchymal Transition genes as E-cadherin, SLUG, SNAIL and vimentin (8).

Moreover, it has been demonstrated that pancreas-specific deletion of Yap in mouse models completely blocked progression from early neoplastic lesions into PDAC, confirming his crucial role in initiation and progression of KRAS mutant PDAC (9).

In their work, Murakami *et al.* showed that ablation of Yap in Kras:Trp53 mutant pancreatic epithelial cells blocked recruitment of MDSCs in favor of major histocompatibility class II (MHCII) positive antitumor macrophages, resulting in reactivation of T lymphocytes, apoptosis of neoplastic ductal cells and tissue regeneration following pancreatitis. In particular, ablation of Yap resulted in significant downregulation of secreted Il-6, G-Csf, GM-Csf, M-Csf, Tnfα and Il-3 in PDAC mouse models. All these cytokines are known to be critical mediators of MDSC polarization and this suggest a crucial role for Yap in promoting MDSC differentiation in PDAC microenvironment. Interestingly, the study also showed that deletion of Yap induced an increase in the percentage of MHCII+F4/80+ macrophages with increased expression of iNos2 and concomitant decrease in Arginase expression, suggesting a switch of tumor associated macrophages (TAMs) from a tumor promoting to tumor-suppressing phenotype.

The clinical relevance of Yap in PDAC progression was further confirmed by the authors with correlation of Yap expression and survival in PDAC patients. By analyses of The Cancer Genome Atlas (TCGA), authors stratified PDAC patients in three groups based on Yap expression levels and demonstrated that high Yap expression level strongly correlated with poorer survival. Interestingly, Yap was also related to MDSC related gene expression profile, with patients classified as having high MDSC gene expression also showing high Yap levels.

Several studies have confirmed the role of Yap in promoting PDAC initiation and progression, in acquisition of resistance to gemcitabine chemotherapy (10) and in promotion of epithelial to mesenchymal transition. However, the study by Murakami *et al.* is the first to demonstrate a key role for Yap in induction of an immunosuppressive microenvironment in PDAC. These findings are particularly interesting considering the disappointing results so far observed with immunotherapy in PDAC if compared to other malignancies. In the area of immune checkpoint inhibitors, both CTLA-4 and PD-L1 inhibitors were investigated in patients with locally advanced or metastatic pancreatic cancer in two clinical trials. A phase II study tested ipilimumab (3.0 mg/kg) activity in twenty-seven patients with advanced PDAC but non objective response was observed (11). In a phase I trial evaluating an anti-PD-L1 antibody (BMS-936559) in patients with different diseases, despite good results obtained in melanoma and lung cancer, no response was achieved in fourteen patients with advanced PDAC (12). A possible explanation of these results is associated with the complex relationship between the tumor, tumor microenvironment and immune system in PDAC. Desmoplastic stroma is a distinctive feature of PDAC and consists of regulatory immune cell populations, activated stellate cells, extracellular matrix (ECM) proteins, and fibroblasts. These cancer-associated fibroblasts (CAFs) represent the most abundant cell type in the tumor stroma. Activation of CAFs leads to the production of ECM components, including collagens, secreted protein acidic and rich in cysteine (SPARC), osteopontin, osteonectin, elastin, tenascin-C, fibronectin, thrombospondin, proteoglycans, hyaluronic acid, and STAT3. In addition, CAFs secrete chemokine ligand 12 (CXCL12) and interleukin 17 (IL-17). These mediators suppress T cells via chemokine receptor 4 (CXCR4) (13). It has been demonstrated that CXCR4-CXCL12 axis may be related to resistance to immune checkpoint inhibitor treatment because the blockade of this signal has a synergistic effect with anti-PD-1 therapy (14). The presence of immunosuppressive cells, such as MDSCs or Tregs, and consequent decreased function of T cells and natural killer (NK) cells in PDAC stroma is another possible reason for failure of immune checkpoint therapy.

In this scenario, new strategies are currently being tested to increase efficacy of immunotherapy in PDAC and overcome resistance to immune checkpoint inhibitors treatment. Combination treatments, including chemotherapy or radiation therapy in addition to checkpoint inhibitors, is one of the strategies currently under study. Indeed, preclinical data showed that chemotherapy agents commonly used in PDAC treatment, such as gemcitabine and oxaliplatin, have significant immune effects such as enhancement of cellular immunity, augmentation of dendritic cell maturation, and reduction

of MDSC and Tregs (15). Another promising strategy is represented by combination of cancer vaccines or cyototoxic T cell stimulators with immune checkpoint inhibitors treatment. For example, combination of GVAX and ipilimumab has been evaluated in advanced PAC in a randomized phase 2 trial, compared to single agent ipilimumab. Combination therapy achieved interesting results, with CA19-9 biochemical response and prolonged patient survival, although the difference was not statistically significant (16). The role of cancer vaccines in initiation of antitumor immune response has been confirmed by a recent neoadjuvant study that assessed the effects of GVAX given with a low-dose of cyclophosphamide to target suppressive Tregs 2 weeks before surgical resection of pancreatic tumors. In this study, the use of an allogeneic granulocyte-macrophage colony-stimulating factor (GM-CSF)—secreting whole-cell pancreatic tumor vaccine (GVAX) was associated with intratumoral tertiary lymphoid aggregates in the majority of resected surgical specimens and these structures were shown to be regulatory, that is, they induced antigen-specific T cells that could still be downregulated by immune checkpoint signals within the tumor, including PD-L1 (17). Ongoing trials are evaluating combinations of GVAX and anti-PD-1 monoclonal antibodies as immune strategies in PDAC in different clinical settings, including neoadjuvant therapy, adjuvant therapy, and metastatic disease.

Considering the results by Murakami *et al.*, inhibition of Yap seems to be another promising strategy to enhance the effectiveness of T-cell checkpoint inhibitors or others immunotherapy in PDAC reducing MDSC and TAM-mediated immune suppression. Despite some challenges in targeting Yap, due to the lack of enzymatic pocket in the molecule, progresses have been made in the development of small molecules that may act as Yap inhibitor, in particular through inhibition of Yap-TEAD complex interactions (18,19). Interestingly, the role of Yap has been recently studied also in other malignanices. In a study on 92 cases of non-small cell lung cancer (NSCLC) YAP was found to be expressed in 66.3% (61/92) cases and predominantly presented in the nucleus. Yap expression in NSCLC was significantly correlated with pTNM stage and lymph node metastasis (P=0.0093) and was also associated with short overall survival, suggesting a role in NSCLC progression (20).

Immunotherapy may represent a promising treatment modality also in PDAC. However, there still remains much to be learned about the pancreatic immune microenvironment and its role in the immune escape of cancer cells. Preclinical and clinical studies have revealed the synergy between immunotherapy and other targeted therapeutics, including using the appropriate costimulatory molecules or cancer vaccines to increase the density of the intratumoral effector T-cells or decrease or inhibit the immunosuppressive cells. In this view, studies that improve our knowledge of tumor microenvironment and relationship between tumor and immune system are eagerly warranted.

Acknowledgements

None.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

References

1. Rahib L, Smith BD, Aizenberg R, et al. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res 2014;74:2913-21.
2. Martínez-Bosch N, Fernández-Barrena MG, Moreno M, et al. Galectin-1 drives pancreatic carcinogenesis through stroma remodeling and Hedgehog signaling activation. Cancer Res 2014;74:3512-24.
3. Topalian SL, Drake CG, Pardoll DM. Targeting the PD-1/B7-H1(PD-L1) pathway to activate anti-tumor immunity. Curr Opin Immunol 2012;24:207-12.
4. Parker KH, Beury DW, Ostrand-Rosenberg S. Myeloid-Derived suppressor cells: critical cells driving immune suppression in the tumor microenvironment. Adv Cancer Res 2015;128:95-139.
5. Gabitass RF, Annels NE, Stocken DD, et al. Elevated myeloid-derived suppressor cells in pancreatic, esophageal and gastric cancer are an Independent prognostic factor and are associated with significant elevation of the Th2 cytokine interleukin-13. Cancer Immunol Immunother 2011;60:1419-30.
6. Murakami S, Shahbazian D, Surana R, et al. Yes-associated protein mediates immune reprogramming in pancreatic ductal adenocarcinoma. Oncogene 2017;36:1232-44.
7. Sudol M, Bork P, Einbond A, et al. Characterization of the mammalian YAP (Yes-associated protein) gene and its role in defining a novel protein module, the WW domain. J Biol Chem 1995;270:14733-41.
8. Thongon N, Castiglioni I, Zucal C, et al. The GSK3β

inhibitor BIS I reverts YAP-dependent EMT signature in PDAC cell lines by decreasing SMADs expression level. Oncotarget 2016;7:26551-66.
9. Zhang W, Nandakumar N, Shi Y, et al. Downstream of mutant KRAS, the transcription regulator YAP is essential for neoplastic progression to pancreatic ductal adenocarcinoma. Sci Signal 2014;7:ra42.
10. Jiang Z, Chen X, Chen K, et al. YAP Inhibition by Resveratrol via Activation of AMPK Enhances the Sensitivity of Pancreatic Cancer Cells to Gemcitabine. Nutrients 2016;8. pii: E546.
11. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother 2010;33:828-33.
12. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 2012;366:2455-65.
13. Mei L, Du W, Ma WW. Targeting stromal microenvironment in pancreatic ductal adenocarcinoma: controversies and promises. J Gastrointest Oncol 2016;7:487-94.
14. Feig C, Jones JO, Kraman M, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. Proc Natl Acad Sci U S A 2013;110:20212-7.
15. Duffy AG, Greten TF. Immunological off-target effects of standard treatments in gastrointestinal cancers. Ann Oncol 2014;25:24-32.
16. Le DT, Lutz E, Uram JN, et al. Evaluation of ipilimumab in combination with allogeneic pancreatic tumor cells transfected with a GM-CSF gene in previously treated pancreatic cancer. J Immunother 2013;36:382-9.
17. Lutz ER, Wu AA, Bigelow E, et al. Immunotherapy converts nonimmunogenic pancreatic tumors into immunogenic foci of immune regulation. Cancer Immunol Res 2014;2:616-31.
18. Liu-Chittenden Y, Huang B, Shim JS, et al. Genetic and pharmacological disruption of the TEAD-YAP complex suppresses the oncogenic activity of YAP. Genes Dev 2012;26:1300-5.
19. Sudol M, Shields DC, Farooq A. Structures of YAP protein domains reveal promising targets for development of new cancer drugs. Semin Cell Dev Biol 2012;23:827-33.
20. Wang Y, Dong Q, Zhang Q, et al. Overexpression of yes-associated protein contributes to progression and poor prognosis of non-small-cell lung cancer. Cancer Sci 2010;101:1279-85.

Cite this article as: Berardi R, Bittoni A. Yes-associated protein and immunosuppressive microenvironment in pancreatic cancer development: a new strategy to improve immunotherapy efficacy? J Thorac Dis 2017;9(7):1798-1801. doi: 10.21037/jtd.2017.06.60

