The Roles of Frequently Mutated Genes of Pancreatic Cancer in Regulation of Tumor Microenvironment

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Abstract
Pancreatic ductal adenocarcinoma has extremely high malignancy and patients with pancreatic ductal adenocarcinoma have dismal prognosis. The failure of pancreatic ductal adenocarcinoma treatment is largely due to the tumor microenvironment, which is featured by ample stromal cells and complicated extracellular matrix. Recent genomic analysis revealed that pancreatic ductal adenocarcinoma harbors frequently mutated genes including KRAS, TP53, CDKN2A, and SMAD4, which can widely alter cellular processes and behaviors. As shown by accumulating studies, these mutant genes may also change tumor microenvironment, which in turn affects pancreatic ductal adenocarcinoma progression. In this review, we summarize the role of such genetic mutations in tumor microenvironment regulation and potential mechanisms.

Keywords
pancreatic ductal adenocarcinoma, local immunity, tumor microenvironment, genetic mutation

Abbreviations
IL, interleukin; MDSC, myeloid-derived suppressive cells; NF-κB, nuclear factor kappa B; NK, natural killer; PanIN, pancreatic intraepithelial neoplasia; PDAC, pancreatic ductal adenocarcinoma; TAM, tumor-associated macrophages; TGF-β, transforming growth factor-β; TME, tumor microenvironment; ULBP, UL16-binding protein.

Introduction
Pancreatic ductal adenocarcinoma (PDAC) is characterized with hidden onset, rapid progression, and dismal prognosis, recognizing as “the king of tumor” among all types of malignancies. Ranking fourth in cancer-related death,¹ it is predicted to become the second commonest cause of death by the year of 2030.² Even in the United States, the 5-year overall survival rate of patients with PDAC is as low as 7%, and less than 20% of patients have opportunity to receive operation.³⁴ Unfortunately, even after radical resection and adjuvant chemotherapy, a large proportion of patients with PDAC relapse within 1 year.⁵ Previous evidence has suggested that the occurrence of PDAC is related to the tumor initiating cells; however, recent studies have found that tumor microenvironment (TME), especially the suppressed local immunity, contributes a lot to the formation of PDAC.⁶

Genetic mutations can be detected in nearly all PDACs, including point mutations, insertions and deletions, amplification, translocations, fusions, and inversions.⁷ Among all mutated genes, KRAS, TP53, CDKN2A, and SMAD4 are most frequently reported and each of them can be found in more than 1 of 3 PDAC cases.⁸⁹ Other frequently mutated genes such as CDKN2B and ARID1A were also detected using the next-generation sequencing.¹⁰ These genes only reported as frequently mutated in recent targeted sequencing implicates that they probably have less allele frequency. The mutated genes may change protein function, resulting in uncontrolled cell proliferation and movement, restrained apoptosis or...
autophagy, impaired DNA repair, and other cancer-related events. In this mini-review, we summarize the roles of the 4 most frequently mutated genes of PDAC in the alteration of TME, particularly in the regulation of local immunity.

Characteristics of Immune Microenvironment in PDAC

The initiation and progression of malignant tumors are affected by multiple factors to form a cancer-friendly microenvironment. First proposed by Ioannides and Whiteside, TME is a complex of extracellular matrix, growth regulators, and other cell components, providing genetic mutation conditions for cancer cells. Besides, it can assist cancer cells in signal transduction, invasion, and distal metastasis. The TME of PDAC contains a large number of compact cell matrix components which are closely related to local immunity including cancer-associated fibroblasts, various types of collagen, hyaluronic acid, and immune cells such as macrophages, dendritic cells, T cells, and B cells. A great quantity of soluble immune-regulatory factors such as cytokines and chemokines are also associated with the locally immunity of PDAC. In PDAC, local immunity is always suppressed, not only providing good conditions for tumor initiation, progression, and distant metastasis of PDAC but also reducing the killing effect on cancer cells.

Effects of Frequently Mutated Genes on Immune Microenvironment of PDAC

Based on the next-generation sequencing, several large-scale genomic studies on PDAC have found a variety of frequently mutated genes including KRAS, TP53, CDKN2A, SMAD4, RNF43, ARID1A, PRM1, GNAS, RREB1, and TGFB2, among which, KRAS, TP53, CDKN2A, and SMAD4 (1 oncogene, 3 tumor suppressor genes) are the most significant ones.

KRAS

As the most frequently mutated gene, KRAS is located on chromosome 12 of human, encoding a small GTP enzyme that mediates downstream signal transduction of growth factor receptor. It plays widespread and essential roles in regulating cell growth, proliferation, differentiation, apoptosis, and other biological processes. KRAS gene mutations can be detected in more than 90% of PDAC. What’s more, the KRAS gene mutations are regarded as the commonest carcinogenic gene mutations. Its main mutation is the amino acid substitution at the 12th position of KRAS protein, that is, glycine (G) is replaced by aspartic acid (D). GI2V and GI2C mutations of KRAS are also found in PDAC, with similar biological effects in activating KRAS protein.

Normally, KRAS protein is associated with GTP-binding activation and GDP-binding inactivation, which enables cell growth cycle to keep in a balanced state. But, the KRAS genetic mutation changes the configuration of KRAS protein, causing the loss of intrinsic GTPase activity and subsequent obstacle of GTP hydrolysis. In addition, its state of continuous activation will lead to aberrant signal transduction, uncontrolled cell proliferation, and inhibited apoptosis. As a result, it may lead to tumor initiation.

In recent years, some genetically engineered mice models expressing oncogenic KRAS mutations have been developed and used to study the role of KRAS in the TME of PDAC. Beside its role in cell proliferation, KRAS also has a marked effect to influence the immune and inflammatory TME. Although the exact mechanism is still unknown, the influence of KRAS in antitumor immune response can be extensively affected by the infiltration of T cells and myeloid-derived suppressive cells (MDSCs). In the formation of pancreatic intraepithelial neoplasia (PanIN), especially during its early stage, injuries induce pancreatic stellate cells and mesenchymal-derived cells to form fibroblasts, leading to fibrin remodeling in the pancreas and enhancing the expression of oncogenic KRAS. The formation and progression of PanIN are also accompanied by the infiltration of immune cells. Studies have shown that the phenotypic changes in pancreatic stellate cells happen before other components of the pancreas. The inactivation of KRAS can alleviate PDAC-associated chronic inflammation. In other solid tumors including lung cancer and colorectal cancer, KRAS mutation was also reported to induce immunosuppressive TME by upregulating PD-L1 expression in cancer cells, inducing regulatory T-cell differentiation, or recruiting MDSCs. In fact, KRAS-mutated cancer cells can exert on all kinds of immune cells by paracrine ways, which was well reviewed by Carvalho et al. For instance, a high level of KRAS activity can produce many factors regulating the maintenance of microenvironment mediators, such as sonic hedgehog, interleukin-6 (IL-6), IL-10, transforming growth factor-β (TGF-β), and prostaglandin E. Notably, these immunoregulatory factors are expressed in an KRAS-dependent manner. In PDAC, sonic hedgehog expressed by tumor cells, while IL-6 is mainly secreted by inflammatory cells but can be significantly induced by KRAS-mutated cells. Interleukin-6 is essential for the development of PanIN in mice and functions as a hub of inflammatory network that can profoundly alter the TME. Prostaglandin E directly acts on carcinoma-associated fibroblasts through prostaglandin receptor 4 and promotes matrix production, which restrains immune cell infiltration and cytokine/chemokine diffusion. KRAS is able to promote ARF6 expression in PDAC cells and causes PD-L1 recycling and immune evasion. In patients with PDAC with diabetes, somatic mutations of immune-related pathway genes such as CD70 and IRF4 are further enriched. Intriguingly, the mutan KRAS protein can also be released from PDAC cells through autophage-dependent ferroptosis and further uptook by tumor-associated macrophages (TAMs), leading to a M2-like switch of these TAMs, which show protumor effects and result in poor prognosis of patients.
TP53

TP53, located on human chromosome 17, encodes the well-known tumor suppressor p53 and is one of the commonest mutant genes of many types of tumors. In addition to its functions of blocking cell cycle and maintaining genomic stability, p53 plays an important role in the regulation of immune microenvironment of PDAC. However, mutant p53 may harbor gain-of-function activities and show various effects on the TME of PDAC. In mouse models, the expression of mutant p53 has been shown to inhibit (but not prevent) the therapeutic response to the recovery of wild-type p53. A great deal of evidence has proved that these mutants of p53 have lost the function of tumor inhibition but have acquired the carcinogenic activity to promote the growth of tumors, depending on its specific mutational statuses.

p53 can modulate immune cells through various ways in PDAC microenvironment. On one hand, wild-type p53 enhances innate immune response by promoting the expression of toll-like receptors on the surface of many types of immune cells particularly TAMs and neutrophils. On the other hand, it can regulate the expression of ULBP1 (UL16-binding protein 1) and ULBP2 at the transcriptional level to enhance the antitumor activity of natural killer (NK) cells. Blocked the negative regulatory effect of MDM2 on p53 by ubiquitin protein ligase 3, Gasparini et al found that the increased p53 expression could boost the number of T cell by enhancing the ability of dendritic cells.

The cytokines in TME are also regulated by p53. Wild-type p53 can inhibit the production of IL-6, cyclooxygenase-2, and inducible nitric oxide synthase through STATs, nuclear factor kappa B (NF-κB), and their signal transduction, finally inhibiting the occurrence and metastasis of tumors. The mutant p53 enhances the expression of NF-κB, leading to severe chronic inflammation and sustained tissue damage. Hayashi and colleagues have shown that mutant p53 regulates TME by facilitating the secretion of vascular endothelial growth factor and activating fibroblasts to promote angiogenesis. The immunomodulatory functions of p53 were comprehensively reviewed by Cui and Guo. TP53 has numerous types of genetic alterations with distinct functions; however, this is not usually considered when investigating the effects of TP53 mutations in PDAC.

CDKN2A

Located on the p21.3 band of human chromosome 9, CDKN2A is an important cell cycle regulator. In sporadic PDAC, up to 90% of the patients are associated with its loss-of-function alteration, mostly with loss of homozygosity, loss of heterozygosity, mutation, and abnormal methylation of promoter. The encoded proteins are p16 (p16/INK4A) and p14ARF, both of which can suppress the initiation of PDAC through restraining cell cycle.

P16 protein is a cyclin-dependent kinase inhibitors. It competes with cyclin D to bind CDK4/CDK6, inhibiting the activities of CDK4 and CDK6. It can also inhibit the cell cycle of G1 phase to S phase. In addition, p16 protein can prevent the pRb phosphorylation by CDK4/CDK6 suppression and increase the number of nonphosphorylated pRb to inhibit cell proliferation. It has been reported that the lower the differentiation grade of PDAC, the faster p16 protein was degraded. Moreover, the metastatic pancreatic cancer shows much higher deletion rate of p16 protein compared with the nonmetastatic PDAC.

P14ARF is specifically produced by a unique exon 1 of CDKN2A, which introduces a changeable reading frame into the downstream exon shared with p16. P14ARF protein induces growth arrest and apoptosis through inhibiting the degradation of MDM2-dependent p53 protein. In addition, p14 inactivation only occurred in the condition of CDKN2A deletion. The expression of p14ARF is driven by another promoter not identical to p16, suggesting that both products of CDKN2A have distinct regulatory mechanisms.

Using integrated analysis, Wartenberg and coworkers found that CDKN2A mutation was significantly related to poor T-cell and B-cell infiltration but enriched Foxp3+ Tregs, leading to remarkably shorter survival in patients with PDAC. A similar study analyzing the public data from The Cancer Genome Atlas demonstrated that nonsilent mutations in CDKN2A rather than KRAS was associated with less infiltration of cytolytic T cells in PDAC. These results highlight the role of CDKN2A mutations in the regulation of tumor local immunity, yet the underlying mechanisms are waiting to be revealed.

SMAD4

SMAD4 is also called DPC4, which is the first human Smad cloned in screening mutations in patients with PDAC. The human Smad4 gene is located on chromosome 18q21.1, containing 11 exons and transcribing 552 amino acids. Smad4 is an intracellular messenger of TGF-β and shows antitumor effect by inhibiting cell growth. SMAD4 mutations can be found in around a half of pancreatic cancer. In case of SMAD4 inactivation due to somatic mutations, PDAC cells may lose their sensitivity to growth inhibition of TGF-β. Moreover, it may suppress the antitumor immune response by enhancing tumor cell invasion and metastasis and by inducing angiogenesis.

Since SMAD4 is a critical downstream factor responsible to the extracellular stimulation of TGF-β, the effects of SMAD4 mutation are to a great extent dependent on the biological functions of TGF-β. Transforming growth factor-β can promote epithelial–mesenchymal transformation, reduce apoptosis of cancer cells, and enhance the immune surveillance. In addition, TGF-β shows widespread immunosuppressive effects in T cells, TAMs, dendritic cells, and NK cells. Therefore, SMAD4 mutation is supposed to counteract these effects. Moreover, Smad4 deficiency in PDAC cells leads to reduced number of S100A8-positive monocytes.

Similar to Kras, Smad4 can also be delivered between cells via exosomes. Pancreatic ductal adenocarcinoma–derived exosomes containing Smad4 was able to recruit MDSCs through
increasing calcium flux and glycolytic activity, resulting in an immunosuppressive TME. The has-miR-494-3p and has-miR-1260a were identified as potential mediators of these processes.

Summary

Pancreatic ductal adenocarcinoma has been a hot research topic because of its rapid progression and high mortality. At present, the emerging immunotherapy is considered as a new hope for PDAC treatment, but it still faces great challenges in the clinical application of PDAC. It is believed that the specific immune microenvironment of PDAC plays predominant role in the response to immunotherapy. The limitation is that many studies did not analyze the mutational status very carefully, so we did not know whether certain mutations were associated with antitumor immunity. The lack of enough cases with certain mutations could be the main reason.

The characteristic TME of PDAC is largely caused by the featured genetic mutations of PDAC cells. These mutations not only influence tumor cells themselves but also regulate stromal cells and extracellular matrix in direct and indirect ways. With our summary, we hope to provide a comprehensive understanding of the association between somatic mutations and local immunity, which will be helpful for the development of immunotherapy and precision medicine for PDAC treatment. However, for this purpose, many efforts are needed to reveal the cross talk between somatic mutations and local immunity in PDAC.

Authors’ Note

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