REVIEW

Is there a CDKN2A-centric network in pancreatic ductal adenocarcinoma?

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Abstract: Pancreatic cancer has a high mortality rate and its incidence has risen rapidly in recent years. Meanwhile, the diagnosis and treatment of this cancer remain challenging. Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, but, currently, no sufficiently effective modalities for its treatment exist. The early diagnosis rate of pancreatic cancer is low and most patients have reached an advanced stage at the time of diagnosis. PDAC evolves from precancerous lesions and is highly aggressive and metastatic. It is essential to understand how the disease progresses and metastasizes. CDKN2A mutations are very common in PDAC. Therefore, here, we have performed a literature review and discuss the role of CDKN2A and some related genes in the development of PDAC, as well as the basis of gene targeting with a correlation coefficient of CDKN2A above 0.9 on the STRING website. It is noteworthy that the interaction of CDKN2A with each gene has been reported in the literature. The role of these genes and CDKN2A in PDAC may provide new directions that will advance the current knowledge base and treatment options since cancer progression is realized through interactions among cells. Our findings provide new insights into the treatment of PADC that can, to some extent, improve the diagnosis rate and quality of life of patients.

Keywords: PDAC, CDKN2A, cell cycle, genes, biomarkers

Pancreatic Ductal Adenocarcinoma (PDAC)

In the industrial age, pancreatic cancer is the fourth most common cause of cancer deaths in the world that is expected to become the second leading cause in the next few years.[1,2] The survival prognosis of patients with pancreatic cancer is worse thananother cancer types due to the low rate of early diagnosis, the high invasiveness and metastatic potential, and the resistance to chemotherapy, as well as the absence of effective treatment for refractory pancreatic cancer.[3,4]

Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer. Approximately 90% of pancreatic solid tumors are PDAC, which are usually diagnosed in the late stage.[5,6] PDAC has one of the worst prognoses among all solid tumors. The median survival time of postoperative patients is 8–12 months and the 5-year survival rate is less than 10%.[7,8] The probability of PDAC metastasis to distant organs is high, mainly in the liver, peritoneum, and the lung. PDAC is usually asymptomatic at an early stage and current screening methods fail to achieve the effectiveness and ubiquity of early diagnosis without invasive surgery, and thus early-stage diagnosis and the standard resection of this cancer are critical to the survival and prognosis of patients.[9,10] Studies have shown that most PDACs are characterized by continuous genetic changes as a result of long-lasting accumulation including of genes,

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such as KRAS and TP53, followed by CDKN2A and SMAD4.[11] More attention is to be focused on understanding the molecular mechanisms of pancreatic cancer development and progression.[12] Therefore, further discovery and identification of predictive biomarkers for therapy are highly necessary for the provision of a rational basis for priority in the treatment of pancreatic cancer patients.[13–15]

CDKN2A

CDKN2A was discovered in 1993 and was a cyclin-dependent kinase inhibitor (CDKI) consisting of three exons. CDKN2A has an alternative splicing exon (El-β) and is located on some 9p21 regions in the chromosome.

CDKN2A encodes four products: p16INK4a, p14 alternate reading frame (p14ARF, mouse p19ARF), cyclin-dependent kinase4 p15 (p15INK4A) and long-chain non-coding RNA (lncRNA) ANRIL (also known as CDKN2B-AS), products involved in cell cycle regulation, differentiation, senescence and apoptosis.[16,17] p16INK4a is one of the important coding products of CDKN2A and an inhibitor of the cyclin-dependent kinase family (CDK), whose amount increases during the aging process of many tissues including islets.[18] The lack of CDKN2A isolates CDK4/6 and prevents its binding to D-cyclin, so that the tumor suppressor protein retinoblastoma (RB) binds to the transcription factor E2F and the loss of protein activity results in cell cycle arrest and cell senescence.[19] The lack of expression of p16INK4a leads to overexpression of CDK4 and proliferation of B cells, rising insulin secretion and causing pancreatic hyperplasia.[20] p16INK4a regulates cyclin D1 expression, and D1/CDK4 is critically involved in cellular metabolism and cell cycle progression, which provides therapeutic potential for inhibiting the progression of pancreatic cancer by cell cycle suppression.[21]

CDKN2A Mutations

In cell immortalization and subsequent transformation in many types of cancer, CDKN2A has high-frequency loss of heterozygosity (LOH) or mutation.[22] CDKN2A involves the function regulation of islets, fat, muscle, liver and immune cells, and even the whole process of uterine development.[23] CDKN2A affects the risk of human vascular disease, including coronary artery disease, aneurysm, ischemic stroke, glaucoma, Alzheimer's disease, endometriosis and periodontitis.[16,24] Additionally, CDKN2A mutations are involved in a variety of cancers.[25] Evidence exists that CDKN2A mutations are strongly associated with the recurrence of melanoma.[26] Significant deletion of CDKN2A can

be used as a biological target in a cell line for early identification of human head and neck squamous cell carcinoma (HNSCC).[27] In early metaplasia, the methylation of the CDKN2A promoter is a very common event in esophageal adenocarcinoma.[28] Dysregulation of CDKN2A in bladder cancer is also frequent.[29] Furthermore, the homozygous deletion of CDKN2A is associated with a more aggressive prognosis of mesothelioma.[30] The mutations of CDKN2A are also involved in the development of primary breast cancer.[31]

CDKN2A in PDAC

Hypermethylation of the CDKN2A promoter was confirmed as a marker of CDKN2A inactivation. Moreover, the hypermethylation of CpG islands in the CDKN2A promoter was found to be mediated by DNA methyltransferase, which is a transcriptional mechanism of silencing tumor suppressor genes, thereby promoting cancer metastasis.[32] CpG methylation-mediated gene inactivation in the CDKN2A promoter region is one of the determinants of malignant tumor development.[33] Transcriptional silencing plays a key role in the development of PDAC, thus CDKN2A is considered a target for this cancer.[34] Promoter methylation provides a new insight into better understanding the role of CDKN2A and opens up new directions for improving the diagnosis and treatment of patients with PDAC.

Various defects in DNA repair genes, such as genes involved in DNA repair pathways or cell cycle regulation (such as CDKN2A), may be involved in the development of PDAC.[35] CDKN2A mutation occurs in early PDAC, and the regions of significant loss or expansion of CDKN2A can be established using specific calculation methods.[35,36] Patients with a CDKN2A mutation in familial atypical multiple melanoma syndrome (also known as pancreatic cancer-melanoma syndrome) have a 20-fold higher risk of pancreatic cancer than individuals without mutations.[37] It is noteworthy that patients with a CDKN2A gene deletion had shorter overall survival (P = 0.002), this indicates that CDKN2A mutations can be used as a prognostic marker for PDAC.[34]

CDKN2A negatively regulates the cell cycle regulator CDK4/6 in the development of PDAC. PDAC has four major driving genes: KRAS, TP53, CDKN2A and SMAD4.[38] Chromosome changes in PDACs lead to the loss of tumor suppressor factors, such as CDKN2A, TP53 and SMAD4.[38] Multiple gene mutations occur throughout the development of PDAC, which are critical for more effective screening and treating of pancreatic cancer and other invasive tumors. Investigations on the mutations of these genes and how

they affect the cell cycle to promote disease progression and metastatic phenotype are thus of substantial significance.

CDK4, CDK6, MYC, TP53 and MDM2 in PDAC

We searched STRING for genes highly correlated with CDKN2A (correlation coefficient more than 0.9 and top 5), including CDK4, CDK6, MYC, MDM2 and TP53 as can be seen in Figure 1.

CDK4

CDK4 is a major regulator of insulin signaling.[39] The activity of CDK4 in mouse islets is critical for the proliferation of differentiated B cells, which is the primary mechanism for regulating islet mass to accommodate the steady-state requirements of insulin secretion, and similar situations may apply to the regulation of adult pituitary cell proliferation.[40] The absence of CDK4 completely inhibited the development of pituitary and islets, and pituitary defects caused male and female infertility.[41] Deletion of CDK4 in islet cell line inhibits Rb hyperphosphorylation, and the CDK4-Rb pathway is critically involved in controlling neuroendocrine cell proliferation.[41] CDK4 activation is essential for the early stage of the carcinogenic process of neuroendocrine cell types and a close relationship exists between cancer development and CDK4 activation.

Cell proliferation is dependent on D-cyclin and CDKs, which are key nodes controlling the progression from the G phase to the S phase. Both cyclin D2 and CDK4 proteins are essential for pancreatic cell growth and replication. CDKN2A inhibits CDK4, which may be a key function of PDAC cell cycle regulation.[42] Furthermore, CDK4 is
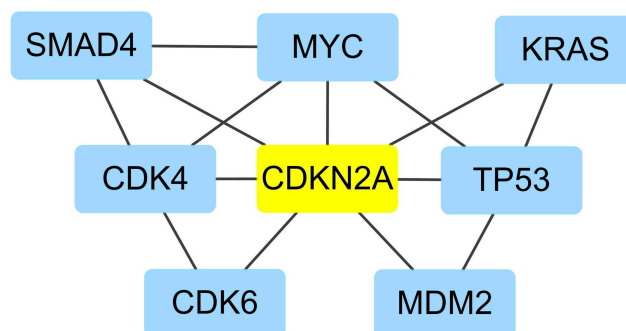

Figure 1 CDKN2A-centric network regulation. Interaction network diagram of CDKN2A on the STRING webpage (correlation coefficient more than 0.9 and no more than 5 interactors) including CDK4, CDK6, MYC, TP53, MDM2 and KRAS, SMAD4. In this picture, CDKN2A, TP53, SMAD4 are tumor suppressor genes, while CDK4, CDK6, MYC, MDM2 and KRAS are oncogenes in almost cancer cells. Lines represent interactions between two genes and we can find out CDKN2A has relationships with these genes in PADC.

involved in hepatic glucose metabolism, and the induction of gluconeogenesis in the liver predetermines the potential for the application of CDK4 inhibitors in the treatment of hyperglycemia in hepatic cancer patients. CDK4 also affects muscle mitochondrial oxidative metabolism, which is essential for maintaining normal physiological functions of the human body.[43] CDK4 affects fat cell function and cell proliferation and metabolism. Its expression level can indirectly influence cell aging and the occurrence and development of various diseases. Future research will address the metabolic function of CDK4 in PDAC and its therapeutic targets in metabolic diseases.[43] Affecting metabolic pathways, CDK4-targeted therapy can regulate the key mechanisms of various cellular processes and environmental cues during the primary, secondary and circulating cancer cell (CTC) transport, thereby controlling PDAC progression.[44] Therefore, CDK4 regulation will have important therapeutic significance for the personalized treatment of pancreatic cancer in the future.

CDK6

CDK6, first reported in 1994 by Meyerson and Harlow, is similar in structure and function to CDK4. It belongs to the same CDKs, which are regulators of D-cyclin kinase and the G1 phase. CDK6 and CDK4 are involved in the regulation of G1 to S cell cycle progression, transcription, differentiation and other biological processes.[45] Activation of CDK4/6 requires binding to cyclin D1, D2, or D3, and the phosphorylation of CDK6 regulates Rb activity, exerting control of the cell cycle, and also blocks the transcriptional regulation of differentiation.[46] The availability of nutrients initiates downstream signals, after which CDK6 is involved in signaling pathways that stimulate metabolism and cell proliferation, promoting cell cycle progression.[47] The transcriptional role of CDK6 is involved in the production of blood vessels in cancers. CDK6 downregulation is associated with the release of Runt-associated transcription factor 1 (Runx1, an important blood-regulating gene, transcription factor required for chromatin opening) in hematopoietic cells.[48] Most of the CDK6 is localized in the cytoplasm, which facilitates the obtaining of more comprehensive knowledge on the role of CDK6 in cell cycle control, differentiation and cancer development.

CDK6 is involved in the negative regulation of cell differentiation. Some cancer cells require CDK6 for their proliferation. CDK6 also promotes the growth of cancer cells and their upregulated expression in various cancer types. The overexpression of CDK6 in lymphoma can be used as a specific therapeutic target for malignant lymphoma.[49] Additionally, the

overexpression of CDK6 in pancreatic cancer promotes the growth and proliferation of pancreatic cancer cells, affects tumor transformation and progression of cell cycle events by participating in the progression of the cell cycle, a process that may be involved in other endocrine cancers.[50] The knockout of CDK6 expression in colorectal cancer COLO320 cells significantly inhibited tumor cell growth, indicates that CDK6 inhibitors present potential therapeutic benefits for colorectal cancer patients, this indicates that CDK6 is a key target for anti-tumor therapy.[51] Inhibition of CDK6 blocks tumor cell proliferation and suppresses the supply of tumors to blood demand, which provides new insights into CDK6 transcription, this also inhibits cell cycle and terminates the progression of pancreatic cancer.[52]

The combination of CDK6 and cyclin D3 (D3-CDK6) plays a unique role in glucose metabolism and promotes cancer cell survival, which may become a strong target for cancer treatment.[53] Clinically, PDACs are particularly sensitive to drugs that reproduce p16IN4a activity. CDKN2A-encoded p16INK4a protein inhibits the kinase activity of CDK4 and CDK6. Therefore, studying the effects of the CDK4/6 combination will increase the positive influence of drugs on patients with PDAC.[47,54,55] Given the wide application and effect of CDK4 and CDK6 inhibitors in clinical trials, the use of D3-CDK6 inhibitors for anticancer treatment is expected to improve the clinical efficacy of anti-CDK4/6 treatment, thus positively affecting the treatment and prognosis of PDAC patients.[53]

MYC

MYC is a promoter and oncogenes which is involved in the regulation of various cellular functions, including cell growth, differentiation, adhesion, migration, invasion and apoptosis.[12] MYC controls metabolic pathways, promotes glucose metabolism, glutamine metabolism, fatty acid synthesis, oxidative phosphorylation, nucleotide synthesis and ribosomal biogenesis.[56] The loss of MYC leads to the misuse of fat as a cell energy source and eventually results in mitochondrial dysfunction.[57] Many genes involved in the glucose metabolism are target genes of MYC, which promotes glucose metabolism reprogramming of cancer cells through different targets, providing bioenergy to ensure cell growth and metabolism the unlimited proliferation of cells.[58,59] MYC-induced cell growth and bioenergy accumulation depend on the production of appropriate ATP in mitochondria.[60] MYC controls glycolysis and thus regulates the overall mitochondrial function,

increasing the resistance of cells to individual metabolic disorders.[61]

MYC selectively activates many genes involved in cellular processes, including DNA replication and transcription, translation, chromatin modification and protein synthesis and degradation. Moreover, MYC coordinates the metabolism and promotes cell growth, enhancing RNA extension throughout the genome and interacting with other transcription factors.[62–65] MYC exists in open chromatin regions in almost all genomes. The level of amplification entirely depends on the pattern of open chromatin in the gene.[66,67] Acute MYC activation causes chromatin changes and promotes gene transcription by inhibiting RNA polymerase promoter pauses.[68] Cancer cells have a stronger ability to replicate and proliferate than normal cells.[69] In rapidly proliferating cells, especially cancer cells, MYC directly regulates protein synthesis and responds efficiently to growth signals.[70] The activation of mutations in MYC induces a normal short-circuit of the cell's mitotic signal, forming a wound that cannot heal and promoting the development of cancer.[36,71] MYC has an indispensable role in cancer and the deletion of MYC inhibited cell proliferation in a human Burkitt's lymphoma cell line.[72] Moreover, MYC is one of the most frequently amplified oncogenes.[73] The abnormal expression of MYC in many cancers, including PDAC, is usually not due to dysfunction of the MYC gene itself, but is caused, for example, by amplification, chromosomal translocation, or upstream carcinogenic signaling disorders, as well as by a loss of tumor suppressor factors.[74,75] MYC is required for the maturation and maintenance of embryonic and adult acinar differentiation, and the activation of MYC signaling induces cancerous changes in pancreatic tissue.[76] MYC overexpression occurs in up to 42% of late PDAC, which is associated with poor clinical outcomes, increased probability of recurrence, worsening disease, and decreased survival in patients with PDAC.[36] In addition, MYC was found to protect pancreatic cancer cells from failure and inhibits their differentiation, indicates that MYC is a critical therapeutic target in PDAC.[77]

MYC is downstream of many growth-promoting signaling pathways, including signaling pathways initiated by growth factor-stimulated receptor tyrosine kinases, T-cell receptors and WNT signaling.[12] MYC is an important downstream effector of KRAS in PDAC. KRAS expression disorder is common in PDAC, and MYC may be involved in the early development and progression of this cancer.[78,79] Aerobic glycolysis is a metabolic feature of invasive cancer, in which MYC promotes the progression of PDAC.[80] The

knockout of the MYC gene reduces LDL expression, lactate production, glucose consumption and alternative approaches that focus on interfering with MYC-mediated downstream effectors may provide new therapeutic avenues for PDAC.[81] Besides, MYC regulates the ability of related stem cells, blocks cellular senescence and differentiation, and coordinates the changes in the cancer microenvironment, including activation of angiogenesis and inhibition of host immune responses. Given that MYC regulates multiple components of the cellular process, further research is required on the MYC-regulated pathways to ensure normal cell function while preventing the occurrence of ineffective cycles.[82]

TP53

More than 50% of the malignant cancers have mutations in TP53, and the frequency of these mutations varies considerably, depending on the specific cancer type. TP53 mutation leads to the loss of its tumor suppressor function, contributing to the development of solid and hematopoietic cancer.[83] PDAC is often associated with dense interstitial fibrosis, which leads to drug resistance, cancer growth and metastasis. TP53 induces lipid accumulation and plays a key role in fibrosis, exerting anticancer effects through a paracrine mechanism in the cancer microenvironment.[84] Evidence exists that TP53 and MDM2 are involved in the AURORA signaling pathway. Hence, affecting the AURORA signaling pathway by influencing TP53 and MDM2 can indirectly lead to changes in the occurrence and progression of PDAC.[85] In the past, many signaling pathways, nutrients and natural products interacted with pathways involved in TP53 and drug resistance.[86]

TP53-positive chronic pancreatitis is considered inefficient in inducing carcinogenesis. On the contrary, in the absence of TP53, the genetic instability affects the latency required for tumor formation, DNA damage, etc., resulting in increased pancreatitis-induced carcinogenesis.[87] In an examination, the expression of TP53 in PDAC tissues was significantly lower than that in normal tissues and benign tissues, the overall survival rate of patients lacking TP53 was poor. The low expression of TP53 in people was associated with an increased risk of PDAC.[88] Mainly due to DNA amplification of genes in PDAC tissues, a high proportion of oncogenes MDM2, MDM4 and WIP1 are overexpressed in cancer cells. These gene changes attenuate the function of the cancer suppressor TP53, which results in TP53 mutation. These changes also contribute to the development of cancer, indicates that the dysfunction of the TP53

pathway may be an important mechanism for the proliferation and progression of PDAC.[89] The deletion of TP53 in mice induces cancer formation and the recovery of its expression level will rapidly resolve the formed carcinoma in situ. This indicates that TP53 has the therapeutic potential to repair cancer tissues and new therapeutic strategies can be developed to combat cancers lacking TP53, which will have a therapeutic impact.[90–92]

Small-molecule activation of TP53 is an attractive therapeutic strategy for the restoration of the wild-type TP53 function in the treatment of PDAC. Based on these strategies, MDM2 inhibitors had a good beneficial effect on TP53 wild-type cancer in vivo and in vitro.[93] Nonetheless, some evidence suggested that TP53 inactivation or dysfunction can directly or indirectly lead to the promotion of tumorigenesis. However, most mutations of TP53 lead to the loss of function, resulting in apoptosis and senescence. Moreover, further validation and functional assessment of these genetic variants, targeting TP53 can also improve the prognosis of patients with PDAC to some extent.[88,94]

MDM2

MDM2 (mouse double microsome 2) is an oncogene found in a locus amplified on the double microsome of a tumor-bearing mouse cell line (3T3-DM) in 1992. The function of TP53 in cells is mainly regulated by its inhibitor, MDM2. The latter directly binds to the N-terminus of TP53 by regulating the position, stability and activity of TP53, and derives TP53 from the nucleus, continuously promoting the degradation of TP53 transforming it into a short-lived protein.[95,96] Cellular stress, such as oncogene activation or DNA damage, disrupts the mutual regulation relationship between MDM2 and TP53, and MDM2 itself is polyubiquitinated and degraded. Meanwhile, the level and activity of TP53 increase, inducing a cellular response to cancer cells, including cell cycle arrest, apoptosis and aging.[97,98] The MDM2 gene contains the TP53 promoter, which is regulated by TP53 transcription, TP53 acts as a transcription factor of MDM2, which induces the synthesis of MDM2.[98] This regulatory mechanism is subject to a feedback loop in which MDM2 inhibits TP53 activity, which in turn regulates MDM2 transcription levels, resulting in a delicate balance between the amount of TP53 and that of MDM2. The two form a negative feedback loop, that is, the automatic adjustment loop.[99]

MDM2 is overexpressed in many tumors, including PDAC, usually due to the expansion of chromosome segments, and its amplification is closely related to tumor

metastasis. MDM2 ubiquitinates and degrades E-cadherin, which is associated with EMT and tumor grade. This is an important step in the development of metastatic tumors.[100] Approximately 50–75% of PDACs carry TP53 mutations, and the MDM2–TP53 pathway in PDAC is frequently affected.[101] A mouse model with a cancer-associated single nucleotide polymorphism had high levels of MDM2 expression, decreased TP53 function and increased tumor incidence.[102] The overexpression of MDM2 inhibited the transcriptional activity of TP53, elevated the proliferation and invasion of cancer cells, suppressed apoptosis and induced resistance to chemotherapy.[103,104] MDM2 was involved in DNA double-strand break (DSB) repair. Delayed the expression of MDM2 in a mouse PDAC model, the cells repair DNA was damaged, caused an arrest of the cell cycle and slowed down the growth of PDAC cells.[105] Furthermore, the over-expression of MDM2 in PDAC was associated with diverse clinicopathological features, such as invasiveness, high differentiation, advanced stage, translocation, recurrence and poor prognosis.[106] Patients with MDM2 expression had a shorter median (OS) and progression-free survival (PFS) than others. Thus, MDM2 expression is an unfavorable prognostic factor for PDAC.[104,107]

The TP53 mutation in PDAC occurs in its late stage, which results in a loss of the DNA binding ability and induces transcriptional activation of the gene. There is a TP53 mutation in PDAC, which may invalidate MDM2 inhibitors. TP53 mutations facilitate the escape of damaged cells from cell cycle checkpoints and their transformation into carcinogens with an increased cancer potential for metastasis and invasion to other organs.[108,109] To avoid infections and the formation of TP53 wild-type cancers, the treatment design is crucial to be based on a comprehensive understanding of the effects of MDM2 on tumors. Hence, the identification of a new TP53-independent therapeutic agent that inhibits MDM2 is highly necessary.[110,111] Different TP53 mutations may have diverse activities and appropriate customized treatments need to be considered along with a timely genetic diagnosis. A better understanding of onco-genes and tumor suppressor genes will lay the foundation for prompt diagnosis and effective treatment of PDAC.

KRAS and SAMD4 in PDAC
KRAS

In many cancers, KRAS is an oncogene.[112] The development of pancreatic ductal adenocarcinoma PDAC is a slow process and most often begins with mutations of the oncogene KRAS

in pancreatic cells.[113,114] About 90% of pancreatic tumors express mutations in KRAS, the earliest genetic changes in most pancreatic cancers.[115,116] KRAS overexpression induces up-regulation of EMT signals such as vimentin and inhibition of E-cadherin, driving the occurrence of early PDAC tumors.[117,118] Some cases with KRAS mutation and TP53 deletion, early PDAC converted to invasive PDAC.[119] KRAS mutations allow cell cycle arrest to enter the aging process and are usually established and maintained by TP53 and its upstream regulators CDKN1A and CDKN2A in combination with activation of retinoblastoma (RB) tumor suppressor pathways.[73,120] The oncogene KRAS plays a central role in PDAC cell proliferation, and its mutation requires CDKN2A, TP53 and MYC are also regulated by KRAS.[117] KRAS mutations depend on CDKN2A inactivation and KRAS mutations are the preferred evolutionary pathway for CDKN2A inactivation.[121] After KRAS mutation, KRAS downstream signal MYC is amplified and CDKN2A inactivation may occur at the same stage.[121] MYC is an important mediator of metabolic changes in pancreatic cancer cells induced by KRAS.[73] Inactivation of TP53 and SMAD4 occurs mainly in true invasive cancers.[122] MYC amplification can induce TP53 and SMAD4 silencing, mutation or loss and promote further development of PDAC.[123,124]

SMAD4

SMAD4 is a core component of the TGF-β signaling pathway.[125] Changes in genes related to the TGF-β signaling pathway are present in almost all PDAC cases. In the advanced stages of cancer, genetic changes may accelerate disease progression, for example by inducing epithelial-mesenchymal transition (EMT).[126] The loss of SMAD4 may prevent the occurrence of EMT.[127] Many studies have shown that SMAD4 deletion or mutation is a negative prognostic factor for PDAC.[128] In cases of SMDA4-deficient PDACs, TP53 usually has a missense mutation.[129] Both TP53 and SMAD4 inactivation occur in the late stages of PDAC, but TP53 mutations occur earlier than SMAD4 loss. TP53 mutations and SMAD4 loss occur mainly in aggressive PDACs and they may be markers of high metastatic potential for tumors.[122,123,130] Both SMAD4 deletions and mutations contribute to the inactivation of SMAD4 and the role of SMAD4 in cancer progression has only been partially reported.[131,132] Some researchers have reported that SMAD4 deletion is more common in early or resectable tumors.[133] Reports show that SMAD4 is lost more frequently in patients with PDAC (72%) with extensive metastatic

disease than in patients with locally destructive disease (35%, p = 0.007).[130] This findings may have potential significance for patients with PDAC and provide new ideas for treatment options to reduce local or distant tumor growth.[129]

Interactions in PDAC

Genomic analysis reveals repeated mutations in many genes in PDAC and the oncogenic point mutations of individual genes aggregate into core molecular pathways.[108] Using targeted sequencing to detect the number of mutation-driven genes and subsequent sequence analysis may be a promising genomic biomarker approach for predicting postoperative and prognosis in patients with PDAC.[134] In previous examinations, gene sequencing confirmed that chromatin remodeling, DNA repair, cell cycle, WNT-β-catenin and NOTCH signaling pathways may be disrupted in PDAC cells. The interaction between these pathways is complex and most genes of these pathways would alter actively or be altered passively.[135,136] CDKN2A and the aforementioned genes participate in the metabolic pathways of cells and can develop effective antimetabolites to target each cancer according to the differences between specific metabolic pathways.[58] Therapy for a specific gene or pathway will have a positive impact on improving the patient's survival prognosis.[136,137]

D-cyclin (D1, D2 and D3) and the associated cyclin-dependent kinases (CDK4 and CDK6) are part of the core cell cycle machinery that drives cell proliferation. CDK4 is involved in the G1-S phase, CDK6 is a regulator of the G1 phase and the product of CDKN2A, p16INK4a, participates in tumor angiogenesis by regulating CDK4/6, promoting cell cycle and participating in metabolism. MYC only amplifies existing transcriptional programs in cells and it is unclear how MYC regulates the ratio between growth-promoting and growth-inhibiting genes to stimulate growth and proliferation.[65] MDM2 and TP53 interact by automatic negative feedback adjustment. Mutations of CDKN2A have been detected in many human cancer types. In this respect, CDKN2A is similar to the typical tumor suppressor gene TP53. TP53 prevents cell cycle progression by increasing the expression of the paralogous gene of the cyclin-dependent kinase inhibitor CDKN2A, CDKN1A.[138] TP53 plays an important role in maintaining genetic stability including DNA repair, cell cycle and apoptosis.[139] MDM2 phosphorylation can affect CDKN2A expression changes.[140] CDKN1A, MDM2, TP53 are included in the TP53 signal pathway in PDAC.[129] In the process of cell division, the major

transcription factor of the TP53 pathway and its cofactor CDKN2A prevent the proliferating cells from repairing the damage by strongly fighting the MYC pathway, or initiate an orderly suicide if the damage cannot be repaired, in order to protect the integrity of the entire tissue.[141,142] TP53 and CDKN2A are often biallelically inactivated in human malignancies, which has a significant impact on treatment.[143,144] MYC induces the inactivation or loss of TP53 by activating the tumor suppressor p19ARF, at this time p19ARF and MYC have a significant carcinogenic synergy.[145] Disruption of the TP53 pathway in PDAC may lose the intrinsic proliferation pathway triggered by MYC activation through mechanisms other than apoptosis inhibition and promote tumorigenesis.[145] MYC can fight growth inhibition mediated by the CDK inhibitor CDKN2A.[146] CDK4 can inhibit the activity of CDKN2A, which is a transcription target of MYC.[146] After CDKN2A deletion, MYC amplification and non-silent mutations and/or deletions are significantly enhanced, which contributes to the metastatic function of PDAC cells.[135,147] Subsequently, MYC regulates the rapid rise of CDK4 mRNA levels through four highly conserved MYC binding sites in the CDK4 promoter, promoting tumorigenicity and cell cycle regulation.[146] Cells are deficient in MYC, the amount of CDK4 protein is reduced and cell cycle progression is delayed.[146] After MYC deletion, the level of apoptotic regulator TP53 was significantly up-regulated and the proliferation rate of cancer cells was slowed down, possibly due to the presence of TP53 mutation.[135] The downregulation of MYC may trigger other cell death mechanisms besides apoptosis, such as autophagic cell death, which is of great significance for the treatment of PDAC.[148]

Conclusion

Of the relationship between CDKN2A and five genes for which we searched on the STRING webpage and KRAS and TP53, a CDKN2A-centric network regulation may exist in PDAC, although the relationships between CDK6 and MYC in PDAC require further research for their better understanding. Early identification of this cancer is of critical importance to better prognosis and survival. Since no specific biomarkers have been identified to date, novel and more sensitive biomarkers need to be discovered and developed to diagnose early this deadly disease.[149,150] Both CDKN2A and these genes involved in the cell cycle or oncogenic transcriptional machinery can be used for tumor therapy by regulating the oncogenic

transcriptional regulation mechanism and cell cycle progression.[151] Understanding the precise cell cycle and metabolic characteristics, as well as the convergent effects of oncogenic pathways in different types of cancer and exploring the relationship between CDKN2A and these genes is critical for determining the optimal combination strategy and using the advances in tumor biology for achieving more effective treatment of PDAC.[58,152]

Data Sharing Statement

- The way we get the network regulation network in this paper includes the following steps.
- Open the STRING website.
- Enter the Protein Name "CDKN2A", select the Organism "Homo sapiens", press the blue button "Select" and press "SEARCH".
- Select the first default option on the website and press the blue button "Continue" of the upper right corner on this website.
- Select the minimum required interaction score "highest confidence (0.900)" and 1st shell of the max number of interactors to show "no more than five interactors", press "UPDATE".
- In the end you will see a CDKN2A-related interaction network picture on this website.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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