73148_Auto_Edited-check.docx

KAI1/CD82 gene and autotaxin-lysophosphatidic acid axis in gastrointestinal cancers

Wang S *et al*. KAI1-ATX-LPA axis in gastrointestinal cancers

Abstract

The $KAI1/CD_{82}$ gene inhibits the metastasis of most tumors and is remarkably correlated with tumor invasion and prognosis. Cell metabolism dysregulation is an important cause of tumor occurrence, development, and metastasis. As one of the important characteristics of tumors, cell metabolism dysregulation is attracting increasing research attention. Phospholipids are an indispensable substance in the metabolism of various tumor cells. Phospholipid metabolites have become important cell signaling molecules. The pathological role of lysophosphatidic acid (LPA) in tumors was identified in the early 1990s. Currently, LPA inhibitors have entered clinical trials but are not yet used in clinical treatment. Autotaxin (ATX) has lysophospholipase D (lysoPLD) activity and can regulate LPA levels *in vivo*. The LPA receptor family and ATX/lysoPLD are abnormally expressed in various gastrointestinal tumors. According to our recent pre-experimental results, KAI1/CD$_{82}$ might inhibit the migration and metastasis of cancer cells by regulating the ATX-LPA axis. However, no relevant research has been reported. Clarifying the mechanism of ATX-LPA in the inhibition of cancer metastasis by KAI1/CD$_{82}$ will provide an important theoretical basis for targeted cancer therapy. In this paper, the molecular compositions of the $KAI1/CD_{82}$ gene and the ATX-LPA axis, their physiological functions in tumors, and their roles in gastrointestinal cancers and target therapy are reviewed.

Core Tip: $KAI1/CD_{82}$ gene inhibits the metastasis of most tumors and is significantly correlated with their invasion and prognosis. According to our recent pre-experimental results, we speculated that KAI1/CD82 might inhibit the migration and metastasis of

cancer cells by regulating Autotaxin (ATX)-lysophosphatidic acid (LPA) axis. However, no relevant research has been reported. To clarify the mechanism of ATX-LPA in KAI1/CD82 inhibition of cancer metastasis will provide an important theoretical basis for targeted cancer therapy, and further research is necessary. In this paper, the molecular composition of KAI1/CD82 gene and ATX-LPA axis, their physiological functions in tumors, and their roles in gastrointestinal cancers and target therapy are reviewed.

INTRODUCTION

The $KAI1/CD_{82}$ gene is an important tumor suppressor gene. $KAI1/CD_{82}$ is a metastasis-related suppressor gene of prostate cancer discovered by Dong $et\ al$[1] in 1995. It is located on human chromosome 1 Lpl1.2 and consists of l0 exons and 9 introns with a length of about 80 kb. The protein encoded by this gene is composed of 267 amino acids residues and has a relative molecular weight of 29600 Da. $KAI1/CD_{82}$ is a member of the transmembrane 4 superfamily (TM4SF). TM4SF protein promotes the interactions between cells and the extracellular matrix, enhances the cohesion between tumor cells, reduces phagocytosis and invasion, and inhibits tumor cell metastasis. Cell dysmetabolism is an important cause of tumor occurrence, development, and metastasis. As one of the hallmarks of cancer, cell dysmetabolism has increasingly attracted the attention of researchers in recent years. Phospholipid is an indispensable substance in cell metabolism and participates in the metabolism of various tumor cells. Phospholipid metabolites have become important cell signaling molecules. Lysophosphatidic acid (LPA) is secreted by platelets, fibroblasts, cancer cells, and fat cells and is a multifunctional "phospholipid messenger". In tumor tissues, LPA induces intracellular signal transduction by binding G protein-coupled LPA receptors (LPARs) on the cell surface and regulates tumor cell proliferation, adhesion, migration, and invasion. Autotaxin (ATX) is a key enzyme catalyzing LPA synthesis. Clarifying the role and molecular mechanism of ATX-LPA and LPARs in cancer invasion and metastasis is necessary. According to our previous experimental results and recent pre-experimental

results, as well as current reports on ATX-LPA, KAI1/CD$_{82}$ might inhibit the cancer cell migration and metastasis by regulating the ATX-LPA axis. The abnormal metabolism of the ATX-LPA axis may be associated with the high metastasis characteristics of cancer. The ATX-LPA axis and their receptors may serve as molecular markers for cancer metastasis and prognosis. Clarifying the mechanism of the ATX-LPA axis in the inhibition of cancer metastasis by KAI1/CD$_{82}$ will provide an important theoretical basis for targeted cancer therapy and further research.

MOLECULAR COMPOSITION OF THE *KAI1/CD$_{82}$* GENE AND THE ATX-LPA AXIS

Molecular composition of KAI1/CD$_{82}$

KAI1 (named after Anticancer Kang Ai) is a tumor-suppressor gene first discovered by Dong *et al*[1] in 1995 on chromosome 11 of rabbit AT6.1 metastatic prostate cancer cells. Later, researchers confirmed that KAI1 has the same structure as the *CD$_{82}$* gene; therefore, it was named KAI1/CD$_{82}$. The 5'-end promoter region of the *KAI1/CD$_{82}$* gene is 735 bp long and rich in CpG island with nine transcription factor-specific protein SPI binding sites, five AP2 binding sites, tcF-1, Myb, and MEP.1 binding sites, which suggests that the gene is regulated by multiple mechanisms[2,3]. KAI1/CD$_{82}$ is located on the cell membrane and is a member of transmembrane 4 superfamily (TM4SF), which comprises four conservative hydrophobic transmembrane domains (TM1–TM4) and one extracellular glycosyl-based binding site. This structure indicates that KAI1/CD$_{82}$, like other TM4SF members, can affect plasma membrane molecular rearrangement, cell aggregation, adhesion, migration, and other physiological and pathological activities through various mechanisms, as well as inhibit the migration and metastasis of various malignant tumors[4].

Molecular composition of the ATX-LPA axis

ATX is a secretory glycoprotein called autocrine motility factor. ATX was first identified in A2058 melanoma cells and induces cell migration through the pertussis toxin G

protein[5]. ATX has phosphodiesterase activity[6], and LPA is catalyzed by lysophosphatidylcholine (LPC)[7]. LPA is a multifunctional "phospholipid messenger" secreted by platelets, fibroblasts, adipocytes, and cancer cells. Although LPA is the simplest phospholipid, it is not a simple biomolecule. LPA has six G-protein-coupled receptors that mediate several physiological and pathological processes, including embryogenesis, wound healing, chronic inflammation, cancer progression, and treatment tolerance[8]. In tumor tissues, LPA binds to LPARs on the cell surface to induce intracellular signal transduction, which in turn regulates tumor cell proliferation, adhesion, migration, and invasion[7]. At present, ATX-LPA target inhibitors are not yet used as a therapeutic measure clinically, and the therapeutic effects of LPA monoclonal antibodies, LPAR antagonists, and ATX inhibitors are still being explored.

ATX is also called extracellular pyrophosphatase/phosphodiesterase (ENPP)$_2$ because of its 47%-55% homology with pc-1/NPP1 and B-10/NPP3 amino acid sequences in the ENPP family. ATX is a multidomain protein[9], and lysophospholipase D (lysoPLD) catalyzes LPA formation[10]. ATX has a slightly U-shaped hydrophobic pocket in the catalytic region, which tends to contain unsaturated substrates, such as unsaturated fatty acids[11], and all five selective splicing isomers have catalytic activity[12,13]. Therefore, its affinity with LPC is strong. Although LPA can be produced by other processes, such as phospholipase A2, Ca^{2+}-independent phospholipase A2, and phosphatidate[14-16], ATX is still the main pathway of extracellular LPA generation.

Serum contains 2-20 μm LPA, and its metabolites extensively affect biological activities inside and outside cells[17]. LPA is one of the smallest glycerophosphatides and comprises three domains: Phosphate head, linker, and lipophilic terminal. The function of the phosphoric head is to activate the receptor; the lipophilic terminal sequence determines its biological activity; and the head and tail are linked by acyl, alkyl, or alkenyl groups[18]. Its free hydroxyl and phosphate groups make LPA more soluble in water than long-chain phospholipids, which likely contributes to its

biological activities. The family of lipid phosphate phosphohydrolases (LPPs) dephosphorylates LPA[19,20].

LPARs are divided into two subfamilies: LPA$_{1-3}$ receptors belong to the endothelial cell differentiation gene (Edg) family, and LPA$_{4-6}$ receptors belong to the purine (P2Y) receptor family[9,21]. LPA$_1$ (Edg$_2$) has 50%-60% amino acid homology with LPA$_2$ (Edg4) and LPA$_3$ (Edg$_7$). LPA$_1$ and LPA$_2$ need to pass through the G$_{i/O}$, G$_{q/11}$, and G$_{12/13}$ signaling pathways, whereas LPA$_3$ passes only through the G$_{i/O}$ and G$_{q/11}$ signaling pathways[22]. The function of G$_{i/O}$ is to stimulate mitotic division through the ras-Raf-MAPK signaling pathway and promote tumor cell survival through the PI3K-Akt signaling pathway[23,24]. LPA$_4$ (P2Y9/GPR23), LPA$_5$ (GPR92), and LPA$_6$ (P2Y5) have 35%-55% homology with amino acids. LPA$_4$ acts through the G$_s$, G$_{i/O}$, G$_{q/11}$, and G$_{12/13}$ signaling pathways and is the only LPAR that activates adenosine cyclase and leads to cyclic adenosine monophosphate elevation. LPA$_5$ plays a role through the G$_{q/11}$ and G$_{12/13}$ signaling pathways, whereas LPA$_6$ plays a role through the G$_{12/13}$ activation of the Rho signaling pathways[22]. The effect of LPARs on tumors depends on the G protein signaling pathway it activates[25].

PHYSIOLOGICAL FUNCTIONS OF THE *KAI1/CD$_{82}$* GENE AND THE ATX-LPA-LPP AXIS IN CANCERS

Inhibition of the KAI1/CD$_{82}$ gene in cancers

Low KAI1 expression accelerates tumor invasion and metastasis[26]. In 2017, a meta-analysis involving 31 studies showed that high KAI1 expression is significantly associated with overall survival (OS) [hazard ratio (HR) = 0.56, 95% confidence interval (CI): 0.47-0.67] and disease-free/relapse-free/progression-free survival (PFS); HR = 0.42, 95%CI: 0.30-0.59) in patients with cancer. In addition, they performed a subgroup analysis showing that KAI1/CD82 is associated with good prognosis in patients with cancer. KAI1/CD82 may be a promising biomarker for predicting the prognosis of patients with malignant tumors, and its biological function has important research value for this topic[27]. The Human Protein Atlas is an outstanding initiative associated

to the Human Proteome Project, which has made available valuable information about the functional and pathological aspects of about 17000 proteins. In particular, they are able to propose scores that suggest the prognostic value of proteins in diseases based on the expression levels of these proteins in healthy and diseased tissues. Considering that only 31 studies were included in the meta-analysis, more studies may be needed in the future to verify whether KAI1 can be used as a prognostic factor. KAI1/CD_{82} may inhibit cell metastasis and migration through two pathways. The first is that KAI1/CD_{82} inhibits cell migration as an initiating signal. However, the possibility of this pathway is low because the simple structure of KAI1/CD_{82} and the lack of corresponding enzymes in the cytoplasm. However, evidence also indicates that KAI1/CD82 may be an initiating signal[28,29]. KAI1/CD_{82} is crosslinked with monoclonal antibody to induce morphological changes and signal transduction[30]. Integrins are also essential for cell adhesion and migration, and KAI1/CD_{82} is associated with several integrins, including α3β1, α4β1, α5β1, α6β1, and αLβ2[31-35], which may also be one of the pathways through which KAI1/CD_{82} inhibits tumor. Epidermal growth factor receptor (EGFR) is a member of the ErbB family. In tumor tissues, the receptors and ligand of the ErbB pathway are overproduced and overactivated. Odintsova *et al*[36] found that KAI1/CD_{82} is correlated with EGFR, ErbB2, and ErbB3 and inhibits the endocytosis of the EGF signaling pathway and EGFR. KAI1/CD_{82} redistributes molecules on the cell membrane surface; KAI1/CD82 overexpression results in the redistribution and aggregation of urokinase-type plasminogen activator receptor (uPAR) into a stable α5β1 complex. Moreover, KAI1/CD_{82} overexpression also results in the redistribution of EGFR and gangliosides in the plasma membrane. However, whether the redistribution of these substances is related to KAI1/CD_{82} tumor inhibition remains unknown[37].

Physiological function of the ATX-LPA-LPP axis in cancers
LPA signals can be roughly divided into three parts, namely, ATX, LPARs, and LPP of extracellular LPA[38,39]. ATX has lysoPLD activity and promotes LPA generation in blood[40,41]. Many tumor cells secrete ATX[42], LPAR expression is higher on tumor cells

surfaces than on normal cells, and LPP expreession is lower in tumor cells than in normal cells. Understanding the metabolic pathway of the ATX-LPA-LPP axis in the tumor microenvironment (TME) is important to study its target therapy.

The TME is produced by tumor cells, such as neuroblastoma[43], glioblastoma[44], liver cancer[45], B-cell lymphoma[46], melanoma[47], kidney cancer[48], thyroid cancer[49], breast cancer and non-small cell lung cancer[50], as well as stromal cells such as fibroblasts and adipocytes[51-53]. How to regulate ATX expression remains unclear. ENPP overexpression may be one of the reasons for ATX upregulation in cancer tissues[54]. The Cancer Genome Atlas shows that ENPP overexpression is present in serous ovarian cystadenocarcinoma (about 33%) and invasive breast carcinoma (about 20%). The $ENPP_2$ gene is overexpressed in hepatocellular carcinoma (HCC) about 20%, lung adenocarcinoma (about 11%), bladder transitional cell carcinoma (about 10%), and head and neck squamous cell carcinoma (about 10%)[13]. Moreover, ATX is involved in the physiological wound-healing response, and ATX levels are increased in some inflammatory diseases[55]. Park et al[56] found that the levels of interleukin (IL)-4, IL-5, and ATX increase in patients with asthma who received bronchoalveolar lavage fluid when stimulated by allergens. ATX induces pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-1β[57,58], NOD receptor family (NLRP3), ATM kinase, ATR protein kinase, and nuclear transcription factor-kappa B (NF-κB)[59]. At present, although ATX research has made some progress, the overall understanding remains limited.

LPA is present in intracellular and extracellular fluids (blood, ascites, follicular fluid, saliva, etc)[56]. In 1989, van Corven et al[60] found that LPA may be involved in cell diffusion and migration. Two years later, Merchant et al[61] found increased LPA levels in malignant colon tumor tissues. LPA may be a simple lipid, but it is involved in all aspects of tumor development; it stimulates proliferative signals[62], prevents growth inhibition and resists apoptosis[63,64], regulates telomerase[64], promotes vascular endothelial growth factor (VEGF)-A and VEGF-C, and induces angiogenesis[65-67]. LPA induces the gene instability caused by reactive oxygen species and stimulates the

production of inflammatory factors, such as COX-2, IL, and TNF-α[68,69]. LPA activates at least three signaling pathways: (1) Promotes phosphoinositol hydrolysis and therefore activates protein kinase C (PKC) and Ca^{2+} mobilization; (2) Promotes the release of guanosine triphosphate (GTP); and (3) Inhibits adenylate cyclase activity. In recent years, the activation of the downstream signaling Ras pathway may promote LPA fibrogenesis[70]. Moreover, MAK-related kinase, as an effector of RhoC, regulates LPA-induced cell invasion through myosin, extracellular signal-regulated kinase (ERK), and P38[71], whereas LPA induces the G12/13-Rhoa-Rock signaling pathway to mediate focal adhesion kinase autophosphorylation and promote tumor cell migration[72]. Furthermore, Lee et al[73] found that LPA interacts with T lymphocytes, B lymphocytes, acidic granulocytes, neutrophils, macrophages, mast cells, dendritic cells, and natural killer cells in the immune system and blood. Currently, no clinical treatment for LPA target is available, and the study of TME's molecular mechanism is helpful to guide clinical treatment.

LPA is hydrolyzed and inactivated by LPPs. Studies have found that LPP1 and LPP3 are reduced in various tumor tissues[74]. LPPs activate ERK signaling by thrombin; induce LPP1 and LPP2 overexpression; and attenuate cell migration, cell differentiation, and angiogenesis[75]. Pilquil et al[76] found that increased LPP_1 expression weakens PLD activation, which is an intermediate substance necessary for LPA to stimulate cell migration. LPP_1 also weakens fibroblast migration. Tanyi et al[77] found that LPP_3 reduces cell apoptosis, decreases the migration ability of transfected LPP_3 cells, and slows down tumor growth in vivo and in vitro.

Comparative analysis of LPAR-mediated signals in tumors

LPA₁: LPA_1 is the most widely expressed Edg LPAR in tissues[69]. LPA signaling through LPA_1 regulates a variety of malignant properties in cancer cells[78]. Murph et al[79] found that LPA_1 downregulates the p53 tumor suppressor gene and weakens its inhibitory effect. Marshall et al[80] found that tumor-suppressor gene Nm23 could inhibit LPA_1 expression. Additionally, Stadler et al[81] found that LPA_1 is a signaling receptor

downstream of fibroblast growth factor receptor 4 (FGFR4) that promotes cell transformation of cells into fibroblasts, which are one of the main components of TME matrix. LPA_1 preferentially binds to Gα Q proteins in tumors to activate PKC. PKC is involved in many cellular processes, including proliferation and metastasis. Valdés-Rives et al[82] found that when the LPA_1/PKCα signaling pathway is blocked, the number of cells is reduced; this finding suggests a correlation between LPA_1 and PKCα in glioblastoma multiforme growth. Stadler et al[81] found that patients with a high expression of the LPA_1 receptor for R388 FGFR4 phenotype are more likely to develop cancer. Lin et al[83] found that LPA_1 signaling mediates tumor lymphangiogenesis by promoting calreticulin expression in prostate cancer. Elevated LPA_1 receptors also contribute to cancer development.

LPA_2: LPA_2 is elevated in tumor tissue[84]. Studies showed that LPA_2 is associated with many human tumors, and the binding of LPA_2 with its ligand, LPA, can activate the LPA signaling pathway and promote cell proliferation and malignant transformation. For example, the high expression level of the LPA_2 receptor in breast cancer suggests poor prognosis[85]. The high expression of LPA_2 mRNA in HCC is related to the low differentiation of cancer cells[86], and the high expression of LPA_2 acceptor in colon cancer cells promotes the acquisition of drug resistance and the failure of anticancer drugs[87]. LPA_2-mediated signaling plays an important role in the enhancement of the chemoresistance of A375 cells treated with anticancer drugs[78]. Malina et al transfected SGC-7901 gastric cancer (GC) cells with LPA_2 expression vector and found that the expression of E-cadherin gradually decreases and the expression of vimentin gradually increases with the increase in LPA_2 level. These findings suggest that LPA_2 is involved in the epithelial-mesenchymal transition (EMT) process of GC cells. GC cells with increased LPA_2 level are likely to metastasize. Dong et al[88] believed that an effective drug that can inhibit LPA_2 gene expression, inhibit GC cell proliferation, and promote apoptosis might be a potential new target for GC treatment. Xu et al[89] found that thyroid receptor interacting protein 6 activates LPA_2 and its downstream signal and

therefore promotes cell adhesion and migration. The carcinogenic mechanism of LPA_2 is still unknown, and most studies have focused on the LPA stimulation of the expression of cytokines, such as IL-6, VEGF, hypoxia-inducible factor 1α, C-MyC, cyclin D1, Kruppel-like factor 5, and COX-2. Moreover, Na^+/H^+ regulatory factor 2 (NHERF-2) may enhance LPA_2 gene expression and other LPA-induced cellular processes[90].

LPA_3: Research found that LPA_3 promotes cancer cell proliferation and metastasis. Xiong *et al* found that the high expression of the LPA_3 protein is considerably correlated with the occurrence and recurrence of epithelial ovarian cancer. Hayashi *et al*[91] and Kitayoshi *et al*[92] found that LPA_3 inhibits tumor cell migration. Sun *et al*[93] found that LPA_3 overexpression is associated with lymph node metastasis and the loss of the expression of estrogen receptor, progesterone receptor, and human EGFR2. Studies found that LPA_3 may be related to the activation of the YAP protein in breast cancer and that LPA_3 overexpression may promote the activation of YAP protein and promote the proliferation and metastasis of breast cancer cells. Fang *et al* found that LPA_3 affects Bcl-2 and Bax expression; therefore, it affects the B cell lymphoma (Bcl)-2/Bax ratio, inhibits the apoptosis of ovarian cancer cells, and promotes the development of ovarian cancer. The vasodilator-stimulated phospho-protein phosphorylation induced by LPA receptor is a key mediator of migration initiation. LPA_3 plays a role in cellular motility and may contribute to cell invasion and metastasis[94].

LPA_{4-6}: LPA_4 may be involved in the invasion and metastasis of breast cancer cells, and the migration and invasion ability may involve the regulation of MMP2 and MMP9 protein expression. Takara *et al*[95] found that LPA_4 is involved in the formation of vascular networks. LPA_4 activation induces the subcellular binding of circumferential actin and enhances the linear adhesion of vascular-endothelial cadherin in endothelial cells. Studies found that LPA_5 knockout cells show high motor activity. The gelatinase spectrum shows that LPA_5 inhibits the activation of MMP2. LPA_5 also inhibits the cellular motility of endothelial cells, which is correlated to the expression level of the

VEGF gene[96]. However, Tsujino *et al*[97] found no mutation in the *LPA$_5$* gene in colon cancer cells, DLD1, SW480, HCT116, CACO-2, SW48, and LoVo. LPA$_6$-mediated tube formation, which reflects the stabilization of barrier integrity, was confirmed by *in vitro* angiogenesis assay. By contrast, LPA$_6$-mediated protective actions are associated with the activation of Src and Rap1 and attenuated by the abrogation of their activities[98]. A considerable correlation between LPA6 and PIM-3 expression levels is also observed in patients with HCC. Furthermore, the biological roles of LPA$_{4-6}$ remain unknown[99,100].

KAI1/CD$_{82}$ GENE AND ATX–LPA AXIS IN GASTROINTESTINAL CANCERS

KAI1/CD$_{82}$ in pancreatic cancer

Pancreatic cancer (PC) is the seventh most common cancer worldwide and causes more than 300000 deaths a year[101]. The 5-year survival rate of PC is only 3%-5%. In the early stages of PC, it directly invades peripancreatic tissues or metastasizes to organs near and far *via* lymphatic and/or blood vessels. More than 80% of patients with PC are initially diagnosed at advanced stages, lose the chance of surgical treatment, and have poor radiotherapy and chemotherapy effects. In 1996, Guo *et al*[102] found that the expression of KAI1/CD$_{82}$ mRNA in early pancreatic tumors (I and II) is significantly higher than that in advanced tumors (III and IV) with lymph node metastasis or distant metastasis ($P < 0.01$), and the KAI1 mRNA level in poorly differentiated tumors is significantly higher than that in moderately differentiated or well-differentiated tumors ($P < 0.05$). Friess *et al*[103] and Xu *et al*[104] also found similar results. Subsequent studies have shown that low KAI1/CD$_{82}$ level is associated with the inhibition of PC cell invasion and metastasis, and the *KAI1/CD$_{82}$* gene may control PC cell metastasis by inhibiting cancer cell invasion and motor function[104-117].

KAI1/CD$_{82}$ protein, a member of TM4SF, has been accepted for its inhibitory effect on tumor metastasis; the mechanism of this effect has not yet been clearly explained, but it may be related to its localization on the cell membrane, extensive glycosylation, and cell-cell and cell-extracellular matrix interactions. Mashimo *et al*[105] found that the loss of p53 leads to the downregulation of the *KAI1/CD$_{82}$* gene and promotes cancer

metastasis. KAI1 may inhibit the metastasis of the PC cells, PANC-1 and Miapaca-2, caused by hepatocyte growth factor (HGF) by downregulating sphingosine kinase (SphK) expression. After they were infected with the *KAI1* gene, the PANC-1 and Miapaca-2 cells induced by HGF had decreased invasive ability in the Boyden chamber assay. KAI1 overexpression in cells leads to the deactivation of SphK and a decreased level of intracellular sphingosine-1-phosphate[112]. Liu *et al*[112] found that KAI1/CD$_{82}$ induces the downregulation of VEGF-C expression through the Src/STAT3 signaling pathway, which may also inhibit the lymph node metastasis of PC. Wu *et al*[118] found that KAI1 induces the expression of the autophagy proteins, LC3 and Beclin1, and further confirmed that KAI1 could induce autophagy in the human PC cell line MiAPACA-2 and therefore promote cell apoptosis and inhibit proliferation. EMT plays an important role in the pathogenesis of PC. KAI1 reverses the expression of EMT-related factors, such as Snail, Vimentin, MMP2, and MMP9 ($P < 0.05$), and inhibits PC cell metastasis and invasion. In conclusion, KAI1 may be a new potential therapeutic target for PC in the future.

KAI1/CD$_{82}$ in HCC

HCC is a common malignant tumor with the second highest mortality rate in China. Rapid intrahepatic and extrahepatic metastases lead to poor prognosis[119]. Zhang *et al* found that the combined detection of KAI1 and VEGF can greatly improve the diagnostic efficiency of HCC treatment. Mu *et al*[120] found that KAI1/CD82 suppresses the HGF-induced migration of hepatoma cells *via* SphK1 downregulation. HGF induces hepatoma cell migration through cellular SphK1 activation. The adenovirus-mediated gene transfer of KAI1 downregulates SphK1 expression and suppresses the HGF-induced migration of SMMC-7721 human HCC cells. Guo *et al*[121] found that the *wTP53* fusion gene and JunB inhibit tumor cell invasiveness and promote tumor cell apoptosis by regulating KAI1/CD$_{82}$ expression. Si *et al*[122] and Yang *et al*[123] found that changing KAI1 expression could alter the migration and invasion ability of MHCC97-H in HCC cells. Xu *et al*[124] found that KAI1 is negatively correlated with tumor grade, venous

invasion, lymph node metastasis, intrahepatic metastasis, and TNM stage and positively correlated with patients' OS. KAI1/CD$_{82}$ may also play an important role in HCC metastasis and prognosis.

KAI1/CD$_{82}$ in GC

GC is one of the most common malignant tumors. Although GC-related morbidity has shown a downward trend in recent years, the mortality rate remains high[125,126]. KAI1 has been studied to identify novel therapeutic targets[127-131]. Ilhan et al[127] and Knoener et al[128] found that KAI1/CD$_{82}$ is negative in all tissues with distant metastasis or tissues in stage IV GC with statistical significance ($P < 0.05$). KAI1 inhibits tumor growth and metastasis and is a prognostic factor for patients with GC. Hinoda et al[129] found that the positive rate of KAI1/CD$_{82}$ in patients with Ia-IIIa GC is 16.6% (8/48), and all patients with IIIb-IVb GC are negative for KAI1/CD$_{82}$ (0%, 0/25; $P = 0.05$). KAI1/CD$_{82}$ is highly expressed in normal gastric epithelial cells. In GC, KAI1/CD$_{82}$ expression decreases with increased tumor differentiation, tumor invasion depth, and lymph node metastasis[132,133]. Guan et al[134] found that reduced KAI1/CD$_{82}$ expression promotes lymph node metastasis and liver metastasis in patients with GC. The detection of KAI1/CD$_{82}$ mRNA expression level can be used as a prognostic target for patients with GC.

KAI1/CD$_{82}$ in colorectal cancer

Colorectal cancer (CRC) is a common malignant tumor, and metastasis is the main cause of its poor prognosis. KAI1 may affect cellular connectivity and may be related to its metastasis. KAI1 may be a new therapeutic target for CRC[135-141]. KAI1 mRNA and protein are increased in early CRC tumors, decreased in late CRC tumors, and no longer expressed in distant metastasis[137-139,142]. Integrin-α3 and TAp73 regulate CRC invasion and metastasis by regulating KAI1 transcription[143,144].

ATX-LPA in PC

The expression of ATX in PC remains unclear, and its molecular biological mechanism has not yet been reported. Ryder et al[145] and Nakai et al[146] found that ATX expression is increased in PC tissues, but it is more increased in chronic pancreatitis or pancreatic cysts than in PC. Quan et al[147] found that TNF-α, NF-κB, Wnt/β-catenin pathway, V-Jun, EGF, and B-FGF are all activated or abnormally expressed in PC tissues, which may provide a direction for future research on mechanisms. LPA activates downstream signaling pathways, such as PI3K/AKT, RAS/ERK, Rho, and Hippo, and promotes PC cell proliferation, migration, and invasion[148,149]. Additionally, LPA is remarkably increased in the serum and ascites[150,151], which suggests that ATX activity is elevated in patients with PC.

ATX catalyzes LPA synthesis from LPC and exerts biological effects through the receptors LPA_{1-6}. Fukushima et al[152] found that the invasion ability of PANC-R9 cells is 15 times that of PANC-1 cells, LPA_1 expression in PANC-R9 cells is remarkably higher than that in PANC-1 cells, and LPA_3 is decreased. Kato et al[153] also found that LPA_1 and LPA_3 play opposite roles in PC cell migration. Tsujiuchi et al[154], Komachi et al[155], and Yamada et al[156] found that LPA_1 induces PC cell migration. Liao et al[151] and Yoshikawa et al[157] found that LPA_2 may induce PC cell migration by enhancing the proto-oncogene K-RAS pathway. However, Komachi et al[155] found that LPA_2 may inhibit PC cell migration through the conjugated G12/13/Rho signaling pathway. Ishii et al[158] conducted a cell activity assay after LPARs were knocked out from PANC-1 cells (PANC-SH4, PANC-SH5, and PANC-SH6 cells). They found that PANC-SH4 and PANC-SH5 enhance cell migration ability, whereas PANC-SH6 inhibits cell migration. Currently, few studies have been conducted on the molecular biology of LPAR and PC, and further research is needed.

ATX-LPA axis in HCC

The main risk factors for HCC are hepatitis virus; alcohol consumption; and metabolic disorders, such as obesity, diabetes, and non-alcoholic fatty liver disease[159]. The abnormal expression of the ATX-LPA axis may cause liver metabolism disorder and

induce steatohepatitis and liver cancer[160,161]. The ATX-LPA axis is currently considered one of the most promising signaling pathways in liver cancer[162]. Watanabe et al[163] found elevated ATX and LPA levels in hepatic fibrosis tissues. Memet et al[159] found that a high expression of ATX in HCC is an independent prognostic factor (HR = 13.70, 95%CI: 3.26-57.62, P = 0.0004), and a high expression of ATX (+3) also increases the risk of death eightfold. Wu et al[164] found that ATX is significantly elevated in Hep3B and Huh7 cells. Park et al[165] found that LPA_1 is significantly elevated in liver cancer. LPA_3 may be highly expressed in HCC tissues through the lPA3-GI-ERK signaling pathway[166]. Enooku et al[86] found that increased LPA_2 mRNA level may be associated with the low differentiation degree of HCC. Okabe et al[167] found that LPA_3 induces the invasion of rabbit RH7777 hepatoma cells. LPA_6 is not expressed in normal tissues but is expressed in liver cancer tissues. Zheng et al[168] found that nuclear receptor coactivator 3 induces the acetylation of histone 3-LYS-27 at the LPA_6 site after HGF treatment and inhibits LPA_6 transcription. High LPA_6 expression promotes HCC proliferation. Lippolis et al[169] found that high LPA_6 expression promotes the development of HCC with poor prognosis. Gnocchi et al[170] and Mazzocca et al[171] found that LPA_6 may be an important therapeutic target for HCC, although LPA_6 overexpression promotes HCC cell growth.

ATX-LPA axis in GC

The role of ATX-LPA axis in GC invasion and metastasis remains to be explored. Zeng et al[172] found that LPA is increased GC tissue samples with peritoneal metastasis (P = 0.046) and is significantly increased in ascites (P < 0.001). Serum LPA decreases after chemotherapy (P = 0.028). PFS and OS are significantly decreased in an ascites LPA > 24000 ng/mL group (P < 0.001). Ramachandran et al[173] and Shida et al[174] found that LPA upregulates SphK1 through the ERK1 signaling pathway. Kim et al[175] found that LPA can induce uPAR to stimulate the downstream signaling pathways, rho-family GTPase, JNK, AP-1, and NF-κB. Budnik[18] found that LPA upregulates human epidermal growth factor receptor 2 expression in GC cells and promotes GC cell

invasion. LPA promotes cell proliferation, but the molecular biological mechanism between LPA and GC still needs further exploration, and LPA may become a new target for GC treatment.

ATX-LPA in CRC

CRC is the fourth leading cause of cancer deaths in the world[176]. Kazama *et al*[177] found that ATX overexpression is associated with tumor angiogenesis in the early stage of colon cancer. LPA may stimulate the proliferation and migration of CRC cells through the EGFR pathway. It may also promote hcT-116 colon cancer cell migration by regulating the cell cycle through the rho-Rock and STAT3 pathways. Whether LPA_1 stimulates colon cancer cell proliferation remains controversial. A study found that HCT116 and LS174T cells knocking out LPA_1 do not affect the spread of cancer cells[178], and DLD cancer cells are affected when they spread[179,180]. LPA_2 promotes the spread and migration of colon cancer by regulating the NHERF-2 pathway[181,182]; therefore, LPA_2 may be one of the therapeutic targets for CRC in the future[183]. Shida *et al*[184] found that LPA_3 mRNA is micro-expressed in normal and tumor tissues. Fukui *et al*[185] found that the expression levels of VEGF-A and VEGF-C are increased in HCT-SH3-3 cells with LPA_3 knockout, and LPA_3 inhibits the metastasis of HCT116 colon cancer cells. Takahashi *et al*[186] found that LPA_4 and LPA_6 inhibit the activities of DLD1 and HCT116 colon cancer cells. Studies on ATX-LPA axis target inhibitors and colon malignancies are still few and require further exploration.

KAI1/CD82 AND ATX-LPA AXIS TARGET THERAPY

KAI1/CD82 target therapy

Most studies have shown that $KAI1/CD_{82}$ inhibits tumor metastasis and migration, but knowledge about $KAI1/CD_{82}$ antibody reagents is still lacking. Custer *et al*[187] found that the Kai1 polyclonal antibody produced by rabbits is expressed similarly in normal tissues of mice and humans and could specifically detect mouse $KAI1/CD_{82}$ protein.

KAI1/CD_{82} is a novel tumor therapeutic target, and more KAI1/CD_{82} antibodies are expected to be developed in the future[188,189].

ATX inhibitors

ATX inhibitors decrease serum LPA levels by more than 95%[190]. Oral ATX inhibitors have better bioavailability owing to their low hydrophobicity and slow degradation *in vivo*[191]. PF-8380 is the first ATX inhibitor to permanently reduce LPA levels *in vivo*. Bhave *et al*[192] and Schleicher *et al*[193] found that PF-8380 reduces lPA-induced inflammation and delays tumor growth for more than 20 d in a mouse model of glioblastoma multiforme. Tang *et al*[194] found that the inhibition of GLPG1690 on ATX enhances the efficacy of chemoradiotherapy in mouse breast cancer models. ONO-8430506 is also a highly effective ATX inhibitor, and the oral administration of 30 mg/kg ONO-8430506 effectively reduces serum ATX and LPA levels in rats[195]. ONO-8430506 in combination with adriamycin delays the growth time of orthotopic 4T1 breast tumors in 60% Balb/C mice by about 10 d and reduces the growth time of 70% tumors by about 17 d[196,197]. Cholera toxin treatment increases the expression of the anti-inflammatory cytokines IL-4 and IL-10 and inhibits ATX mRNA[198], and the knockdown of ATX mRNA inhibits the growth of Hep3B and Huh7 hepatoma cells[199]. Gupte found that ATX inhibitors, such as 4-pentadecylbenzylphosphonic acid, reduce plasma LPA levels by 50%[200]. Plasma LPA in ATX-KO mice lacking dominant heterozygosity is reduced by 50%. ATX inhibitors have not shown remarkable side effects to date.

LPA monoclonal antibody and LPA receptor antagonist

Antibody interventional therapy is superior to traditional therapy, and its antibody bioavailability and receptor binding are longer than other therapies[201]. Goldshmit *et al*[202] found that monoclonal antibody B3 can reduce inflammation and glial cell death and improve neuronal function. Monoclonal antibody B3, also known as lpathomab,

reduces IL-6 expression and the lesion area and has improved function in a mouse model of traumatic brain injury[203].

Many LPA receptor antagonists have been found, but few work *in vivo*. LPA receptor antagonists are divided into lipid and small-molecule inhibitors, which are derived from fibrosis-model studies[204]. BrP-LPA, a pan-LPAR antagonist, was used to treat breast MDA-MB-231 cancer cells[205]. Through LPAR2, BrP-LPA may also sensitize vascular endothelial cells in mouse GL-261 glioma cells to improve malignant glioma response to radiation therapy[193]. LPA accelerates pulmonary fibrosis through LPA$_1$, and the LPA$_1$ antagonist AM966 can inhibit bleomycin-induced idiopathic pulmonary fibrosis. Zhao *et al*[206] found that Ki16425 (LPA$_1$ and LPA$_3$ antagonist) and ono7300243 (LPA$_1$ antagonist) completely blocks LPA-induced actions. Recently, lysophospholipid *GPCR* genes have been used to develop receptor subtype-selective agonists and antagonists. The discovery of FTY720, a novel immune modulator, along with other chemical tools, has provided a means of elucidating the functions of each lysophospholipid GPCR on an organ and the whole body level[207]. In some cancers, targeting LPAR$_5$ is considered a good option against cancer development[87,208]. LPAR$_5$ antagonist TCLPA5 attenuates the proliferation and migration of thyroid carcinoma[209]. In addition, the loss of LPA$_5$ in mouse B16-F10 melanoma results in fewer lung metastases[210], which suggests that the drug inhibition of LPA$_5$ can also control melanoma-mediated metastasis. MP-LPA analogs exhibit an unanticipated pattern of partial agonist/antagonist activity for the LPA G protein-coupled receptor family and the intracellular LPA receptor peroxisome proliferator-activated receptors-γ[211]. Currently, all are based on LPA1, LPA2, or LPA1/3 dual antagonists[204]. However, the development of PAN-LPA receptor antagonists may be a more effective approach owing to the complexity of LPAR signals[212].

CONCLUSION

This paper systematically reviewed the physiological functions of the *KAI1/CD$_{82}$* gene and the ATX-LPA axis in tumors, as well as their roles in digestive system tumors and

targeted therapies. The results demonstrate that KAI1/CD$_{82}$ is indeed an important inhibitor of tumor metastasis. Further elucidation of the molecular mechanism and regulatory network of KAI1/CD$_{82}$ and the inhibition of tumor metastasis is needed to discover the molecular markers of pancreatic tumor metastasis, adopt effective strategies to treat PC and prevent PC metastasis, and provide a new approach for the diagnosis and treatment of patients with refractory PC. Although the ATX-LPA axis is considered an important target of cancer, its clinical application is still faced with obstacles. LPA degrades quickly in the body, and many other factors, such as diet, smoking, and alcohol consumption, can affect the detection results. Other lipids may also generate LPA during extraction, storage, and detection. Therefore, many technical problems need to be overcome in LPA detection. In recent years, clinical trials on the ATX-LPA axis have begun. LPA monoclonal antibodies, LPA receptor antagonists, and ATX inhibitors may become feasible treatment measures. Moreover, ATX-LPA axis-targeted therapy may affect the efficacy of existing chemical drugs. Therefore, an in-depth exploration of specific biomarkers related to LPA activity should be conducted to track disease progression during LPA treatment and ensure the rational application of drugs.

73148_Auto_Edited-check.docx

PRIMARY SOURCES

1   www.nature.com
    Internet
    60 words — 1%

2   Xiaoyun Tang, Matthew G.K. Benesch, David N.
    Brindley. "Role of the autotaxin–lysophosphatidate
    axis in the development of resistance to cancer therapy",
    Biochimica et Biophysica Acta (BBA) - Molecular and Cell
    Biology of Lipids, 2020
    Crossref
    58 words — 1%

3   www.dovepress.com
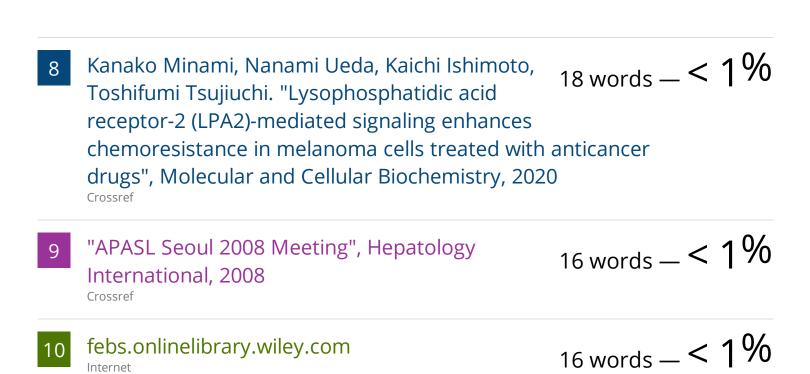   Internet
    44 words — 1%

4   www.ncbi.nlm.nih.gov
    Internet
    39 words — 1%

5   insights.ovid.com
    Internet
    33 words — 1%

6   Joanna Gajewiak, Ryoko Tsukahara, Yuko
    Fujiwara, Gabor Tigyi, Glenn D. Prestwich. "
    Synthesis, Pharmacology, and Cell Biology of -2-Aminooxy
    Analogues of Lysophosphatidic Acid ", Organic Letters, 2008
    Crossref
    22 words — < 1%

7   www.oalib.com
    Internet
    20 words — < 1%

8   Kanako Minami, Nanami Ueda, Kaichi Ishimoto, Toshifumi Tsujiuchi. "Lysophosphatidic acid receptor-2 (LPA2)-mediated signaling enhances chemoresistance in melanoma cells treated with anticancer drugs", Molecular and Cellular Biochemistry, 2020
Crossref

18 words — < 1%

9   "APASL Seoul 2008 Meeting", Hepatology International, 2008
Crossref

16 words — < 1%

10   febs.onlinelibrary.wiley.com
Internet

16 words — < 1%

11   Y.L. Gong, C.J. Tao, M. Hu, J.F. Chen, X.F. Cao, G.M. Lv, P. Li. "Expression of Lysophosphatidic Acid Receptors and Local Invasiveness and Metastasis in Chinese Pancreatic Cancers", Current Oncology, 2012
Crossref

14 words — < 1%

12   Xu Liu, Xiao-Zhong Guo, Wei-Wei Zhang, Zhuo-Zhuang Lu, Qun-Wei Zhang, Hai-Feng Duan, Li-Sheng Wang. "KAI1 inhibits HGF-induced invasion of pancreatic cancer by sphingosine kinase activity", Hepatobiliary & Pancreatic Diseases International, 2011
Crossref

13 words — < 1%

13   www.pubfacts.com
Internet

13 words — < 1%

14   Www.intechopen.com
Internet

12 words — < 1%

15   hdl.handle.net
Internet

12 words — < 1%

16   link.springer.com