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Review and Application of Integrin Alpha v Beta 6 in the Diagnosis and Treatment of Cholangiocarcinoma and Pancreatic Ductal Adenocarcinoma

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Abstract

Integrin Alpha v Beta 6 is expressed primarily in solid epithelial tumors, such as cholangiocarcinoma, pancreatic cancer, and colorectal cancer. It has been considered a potential and promising molecular marker for the early diagnosis and treatment of cancer. Cholangiocarcinoma and pancreatic ductal adenocarcinoma share genetic, histological, and pathophysiological similarities due to the shared embryonic origin of the bile duct and pancreas. These cancers share numerous clinicopathological characteristics, including growth pattern, poor response to conventional radiotherapy and chemotherapy, and poor prognosis. This review focuses on the role of integrin Alpha v Beta 6 in cancer progression. It addition, it reviews how the marker can be used in molecular imaging and therapeutic targets. We propose further research explorations and questions that need to be addressed. We conclude that integrin Alpha v Beta 6 may serve as a potential biomarker for cancer disease progression and prognosis.

Keywords

integrin αvβ6, cholangiocarcinoma, pancreatic ductal adenocarcinoma, imaging, therapeutic target, biomarker

Abbreviations

ARF6, ADP ribosylation factor 6; BRCAm, BRCA1/2 mutations; BTC, Biliary tract cancer; CA19-9, Carbohydrate antigen 19-9; CAR-T, Chimeric antigen receptor T-Cell; CCA, Cholangiocarcinoma; CEA, Carcino-embryonic antigen; CK7, Cytokeratin 7; CK19, Cytokeratin 19; CTGF, Connective tissue growth factor; CTLA-4, Cytotoxic T-lymphocyte antigen-4; DCs, Dendritic cells; DDR, DNA damage repair; EGFR, Epidermal growth factor receptor; EMT, Epithelial-Mesenchymal Transition; Eps8, Epidermal growth factor receptor pathway substrate 8; ERK, Extracellular regulated protein kinases; FGFR, fibroblast growth factor receptor; FLR, Future liver remnant; FOSL1, Fos-like antigen-1; HCC, Hepatocellular carcinoma; HCCA, Hilarcholangiocarcinoma; HER2, Human epidermal growth factor receptor 2; (HMG)-CoA, Hydroxy-3-methylglutaryl; ICC, Intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitor; IDH, Isocitrate dehydrogenase; ITGB6, Integrin β6; LT, Liver transplanation; MAPK, Mitogen-activated protein kinase; MMP-9, Matrix metalloprotein-9; MMR, Mismatch repair; MSI, instable microsatellite; MUC4, Mucin genes 4; NIRF, near-infrared fluorescence imaging; OS, Overall survival; PanIN, pancreatic intraepithelial neoplasia; PD-1, Programmed cell death 1; PDAC, Pancreatic ductal adenocarcinoma; PD-L1, Programmed cell death ligand 1; PDT, Photodynamic therapy; PET, Positron emission tomography; PHC, Perhilar cholangiocarcinoma; PODXL2, Podocalyxin like 2; PSCA, Prostate stem cell antigen; PT, Phototherapy; RAC1, Ras-related C3

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botulinum toxin substrate 1; SPECT, Single-photon emission computed tomography; TGF-β, Transforming growth factor-β; TME, Tumor microenvironment

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Introduction

Cholangiocarcinoma (CCA) is an aggressive malignant tumor that originates in bile duct epithelium. It is a rare cancer that is classified based on its anatomic location within the bile duct tree, as follows: intrahepatic, perihilar, or distal CCA. Perihilar CCA (PHC) is the most common CCA type, with an annual incidence of approximately 2/100 000 in western countries.[1] The three CCA subtypes have different etiologies, risk factors, prognoses, and clinical therapeutic management.[2] CCA is highly fibroproliferative, supported by a dense tumor microenvironment (TME), and has significant genetic heterogeneity, which contributes to its therapeutic resistance. Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive malignancies, accounting for 80% to 90% of all pancreatic cancer cases. Despite its low incidence, PDAC is the seventh leading cause of global cancer mortality.[3] PDAC prognosis is poor due to its complicated and multifactorial nature and overlapping symptoms. The biliary tract and pancreas share a common embryonic origin[4]; therefore, preneoplastic and neoplastic lesions exhibit similar molecular, histological, pathophysiological, and clinicopathological features.[5] In addition, these features contribute to the lack of reliable biomarkers and detection methods for early diagnosis.[6] Thus, the mortality-to-morbidity ratio of pancreatic cancer has not significantly changed in the past few decades.[7] The PDAC 5-year survival rate is less than 5%.[8]

CCA and PDAC treatment options are limited due to the following reasons. In the early disease stage, nerve infiltration, peripheral tissue, and distant metastasis occur; and in the late disease stage, effective non-surgical treatment remains elusive. When feasible, hepatectomy is the preferred treatment for CCA, followed by systemic chemotherapy with capecitabine,[9] but only 30% of cases can be completely removed by surgery. Surgical intervention is classified into two types. One is extended hemi-hepatectomy combined with extrahepatic bile duct resection, which requires strict preoperative evaluation and patients to be in good condition. The other is minimal-invasive resection which has poor effect.[10,11] In other cases, systemic chemotherapy with gemcitabine and cisplatin is usually the first-line treatment option, but the prognosis is still poor.[12] Whether neoadjuvant chemotherapy can improve the survival rate is still unclear. Liver transplantation (LT) can avoid an R1 resection and an inadequate future liver remnant (FLR), but the actual benefits of LT still need more evidence to support. In patients with very early iCCA, upfront LT may be beneficial, while for patients with late unresectable tumor, neoadjuvant chemoradiation is needed.[13]

Targeted therapy, which can be used alone or in combination with chemotherapy, has been utilized as a second-line treatment to enhance patient survival. Many potential molecular targets have been discovered in recent years, including fibroblast growth factor receptor (FGFR),[14] epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2),[15] metabolic regulators such as isocitrate dehydrogenase 1 and 2 (IDH1/2), BRAF, transcription factor Fos-like antigen-1 (FOSL1).[16]

In addition, immunotherapy methods are also being studied. CCA is considered as "immune cold" due to its low and medium tumor mutation load.[17] Furthermore, the TME promotes immune escape and inhibits immune responses. In this case, immune checkpoint inhibitors against programmed cell death 1 (PD-1),[18,19] programmed cell death ligand 1 (PD-L1),[20] cytotoxic T-lymphocyte antigen-4 (CTLA-4) and new therapeutic methods such as tumor vaccines[21] and chimeric antigen receptor (CAR) T-cells[22] can enhance antitumor activity.[23–25]

Chemotherapy and surgery are the main treatment options for PDAC. However, only 20% of patients were eligible for surgery at the time of diagnosis.[7] Most PDAC patients have distant metastasis at the time of diagnosis, and major surgery to remove the main primary lesion is unlikely to improve the prognosis. At the same time, patients must be healthy enough to undergo this big operation and recuperate from it.[26]

In the past decade, two new combination therapies have become the first-line treatment for patients with advanced PDAC. The first is FOLFIRINOX, which stands for 5-fluorouracil (5-FU), leucovorin, irinotecan and oxaliplatin. The second is a combination of gemcitabine and an albumin nanoparticle conjugate of paclitaxel (nab-paclitaxel).[27,28] Patients having surgery usually undergo adjuvant chemotherapy after surgery to eradicate PDAC cells that escape from the original tumor during resection. However, because many patients are unable to bear adjuvant treatment due to surgical complications, systemic preoperative (neoadjuvant) therapy is currently used.[29,30] Studies have shown that neoadjuvant chemoradiotherapy increases overall survival (OS) when compared to postoperative adjuvant therapy.[31]

Despite the progress of surgical methods and the emergence of various chemotherapy regimens, the poor prognosis of PDAC has not improved in the past decades, and there is an urgent need to explore new therapeutic strategies. Therefore, targeted therapy and immunotherapy came into being. The realization of targeted therapy for PDAC patients can be summarized into three methods. First, dysregulated oncogenes such as KRAS, NRG1 and NTRK and related molecules can be suppressed. Second, inactivated tumor suppressors or regulatory related molecules such as TP53, CDKN2A, and Smad4 can

be reactivated. Finally, genes such as KDM6A and BRCA can maintain the structural stability and physiological functions of normal chromosomes, and have defects in some PDAC patients, so they can be used as potential targets to precisely eliminate these abnormal tumor cells.[32] In addition, immunotherapies such as CAR-T,[33–36] antibody drug conjugates,[37,38] and immune checkpoint inhibitors also show the potential to accurately target tumors. Due to the complex immunosuppressive TME of PDAC, tumors are protected from effective cytotoxic immune responses. Therefore, the therapeutic utility of immunotherapy as a PDAC treatment is still limited.[39]

The future direction of precision oncology in CCA and PDAC will still focus on target genes and related signaling pathways, and its research will also aid in the identification of curable patient subgroups. Targeted therapy will certainly provide diverse therapeutic strategies for CCA and PDAC and improve their poor prognosis.

Targeted agents for FGFR2 fusions and IDH1 mutations have been developed. In addition, EGFR inhibitors have been combined with first-line chemotherapeutics. However, the treatment results have been poor. Research on CCA-related mutations within EGFR and BRAF has been conducted[40]; however, the prevalence of these mutations in CCA is extremely low, which highlights the need for identification of novel therapies and therapeutic targets. There are ongoing trials investigating immune checkpoint inhibitor (ICI) monotherapy. So far, the results prove limited efficacy; however, in mismatch repair (MMR)-deficient and instable microsatellite (MSI)-high CCA, ICIs have proven effective.[41] Studies have suggested a link between BRCA1/2 mutations (BRCAm) and BTC response to ICI therapy. It is hypothesized that, in addition to BRCA, other DNA damage repair (DDR) pathway genes found in BTC may impact therapy response and should be further investigated.[42]

Furthermore, the search for new biomarkers continues as they could potentially serve as markers for early cancer diagnosis, which could improve patient survival rates. Carbohydrate antigen 19-9 (CA-199) and carcino-embryonic antigen (CEA) are used as clinical markers for monitoring PDAC and CCA prognosis in patients following treatment. However, the markers are expressed in benign conditions such as pancreatitis and cholangitis; and increased expression is not observed in early disease. These markers, therefore, have low sensitivity and specificity.[43,44] Prostate stem cell antigen (PSCA) expression is highly associated with PDAC, and labeled probes targeting PSCA on the surface of pancreatic cancer cells have been used for targeted imaging; however, studies of PSCA in CCA have been conducted.[45] Mucin genes 4 (MUC4) is overexpressed in the majority of pancreatic cancers, and the use of this marker would allow for the distinction between pancreatitis and pancreatic cancer. However, MUC4 is also overexpressed in pancreatic cysts. Recently, MUC4 has been identified as a novel tumor-associated antigen for pancreatic cancer immunotherapy.[46,47] The expression of cytokeratin 7 (CK7) and cytokeratin 19 (CK19) is elevated in cancer, and their expression could be used to predict ICC prognosis.[48] Integrin Alpha v Beta 6 (αvβ6) is not expressed in islet, acinar or hepatocellular carcinoma (HCC) cells, but is expressed at low to moderate levels in the normal pancreatic duct and biliary duct epithelial cells,[49] as well as significantly in PDAC and CCA.[50,51] Consequently, integrin αvβ6 represents a potential therapeutic target.

Integrins are a family of cell adhesion molecules composed of two non-covalently linked heterodimer subunits, α and β. Integrins mediate cell-cell and cell-extracellular matrix adhesion as well as regulate multiple signaling pathways that promote tumor cell proliferation, migration, survival, differentiation, invasion, and metastasis.[52,53] Integrin αvβ6 was first reported in the 1990s. Among them, the αv subunit is encoded by the gene CD51,[54] which is located at 2q31-q32, while integrin β6 (ITGB6) subunit has been shown to be encoded by beta gene located at 2q24-q31.[55] The αv subunit binds to β1, β3, β5, β6 and β8 subunits, whereas the β6 subunit only binds to the αv subunit, which is highly specific.[52] Subunits αv and β6 contain three domains: extracellular domain, transmembrane domain and intracellular domain. The extracellular and transmembrane domains participate in the activation, adhesion and epithelial-mesenchymal transition (EMT) of transforming growth factor-β (TGF-β) through 11 amino acids at the carboxyl terminus, while the cytoplasmic domain affects proliferation, matrix metalloproteinases (MMPs) production, migration and survival.[56–58]

Unlike the large majority of integrins, αvβ6 is rarely expressed in healthy adult epithelial cells; however, it is upregulated during embryogenesis, tissue repair, and carcinogenesis.[59] Integrin αvβ6 is overexpressed in a variety of cancers, including cholangiocarcinoma,[50] and breast,[58] gastric,[60] pancreatic,[61] colorectal,[62,63] ovarian,[64] and endometrial cancers.[65]

It has been found that elevated integrin αvβ6 expression is associated with a poor cancer prognosis, which may be related to EMT, tumor cell invasion and increased metastatic potential. At present, for digestive system epithelial cancer, a large number of studies focus on the expression and role of integrin αvβ6 in colorectal cancer.[66–68] However, some studies have found that almost all PDAC patients have integrins αvβ6 positive expression,[69] and some studies have shown that because integrin αvβ6 expression was significantly up-regulated in CCA tissues but not in adjacent non-tumor tissues or liver derived tumors, it can be used as an immunohistochemical marker for the diagnosis and differential diagnosis of CCA.[50,70] Therefore, although limited research has been done on it, integrins αvβ6 may serve as potential molecular markers for the differentiation, diagnosis, treatment and prognosis of CCA and PDAC. These features will be reviewed in this essay.

Integrin αvβ6 Signaling Pathway in Promoting CCA and PDAC Progression

Integrin αvβ6 and TGF-β1

Recent studies on malignant tumors indicate that transforming growth factor-β1 (TGF-β1) may play an essential role in tumor genesis and development by promoting tumor angiogenesis, invasion, EMT, and immune escape.[71–73]

TGF-β receptor activation induces signal transduction via the formation of Smad complexes that are translocated to the nucleus, as well as via non-Smad pathways, including Erk 1/2, RAS and MAPK.[57] Smad-dependent signaling pathways can suppress tumors by inhibiting cell cycle activation, promoting epithelial cell apoptosis, and maintaining genomic integrity.[74] Alternatively, Smad4 mutations have been identified in PDAC and various types of CCA.[75,76] TGF-β pathway mutations were discovered in greater than 50% of the patients with PDAC. Therefore, tumor inhibition is frequently lost due to the inactivation on Smad4-dependent TGF-β signaling.[76–78]

TGF-β1 binds to TGF-β1 LAP and forms an inactive complex. LAP-β1–binding to integrin αvβ6 participates in TGF-β1 maturation and activation. Activated TGF-β1 plays key roles in many activation pathways in vivo.[79] TGF-β1 also plays a vital role in the maintenance of integrin αvβ6 expression in epithelial cells and activates integrin αvβ6 transcription in the regulatory region of promoters, thereby increasing the integrin αvβ6 expression.[80] Integrin αvβ6 and TGF-β1 have a balanced relationship, and when this balance is disrupted, many diseases can occur.[81]

Tumors evade the host immune response by secreting immunosuppressive cytokines into the TME, such as TGF-β1. Thepmalee[82] detected the TGF-β1 ligand in dendritic cells (DCs) supernatant and bile duct cancer cells. Other studies[83] have shown that TGF-β1 drives cell migration in CCA by inducing a transition from epithelial to mesenchymal cell phenotype without affecting cell proliferation; thus, increased TGF-β1 gene expression is associated with a poor prognosis of CCA.[84] Blocking the TGF-β1 receptor on DCs, with specific neutralizing antibodies, increases the cytolytic ability of DC-activated effector T cells.[82] Connective tissue growth factor (CTGF) is a cell-matrix protein that mediates cell-matrix interactions through various integrin receptor subtypes. CTGF and the integrin αvβ6 protein are highly expressed in the ductal reaction of human liver cirrhosis and cholangiocarcinoma. A study by Pi[85] discovered that integrin αvβ6 could bind to CTGF, mediate oval cell adhesion to CTGF and fibronectin matrices, and promote TGF-β1 activation in vitro..[86] These results suggest that TGF-β1 is crucial for tumor evasion, and integrin αvβ6 may influence the TGF-β1 pathway to promote tumor progression.

The interaction between integrin αvβ6 and TGF-β1 merits additional research. An important research question could address how the balance between integrin αvβ6 and TGF-β1 and their expression could be maintained in Smad-deficient PDAC and CCA. In addition, research should focus on whether enhancing the Smad-dependent TGF-β1 signaling pathway in Smad-maintained PDAC and CCA inhibits tumor formation?

Integrin αvβ6 and Rac1

Rac1, a member of the small GTPase Rho family, is highly expressed in numerous cancer cell lines and is involved in various cellular processes, such as cytoskeletal recombination and gene transcription.[87,88] Furthermore, Rac1 regulates several downstream effector molecules associated with tumor aggressiveness, such as MMP-9 and uPA, making it a central regulator of tumor malignancy.[89,90]

Tod[91] discovered that Eps8 overexpression promotes integrin αvβ6-dependent tumor invasion while inhibiting integrin αvβ6-dependent TGF-β1 activation in PDAC. The study revealed an inverse relationship between tumor cell migration expression and TGF-β1 activation, and this function could be affected by the presence or absence of Eps8. Eps8 regulates tumor invasiveness by Rac1 activation, which inhibits stress fiber formation and conformational alterations in the TGF-β1-LAP complex, thereby antagonizing the Rho pathway. The Eps8/Abi1/Sos1 tricomplex was discovered to be a crucial regulator of integrin αvβ6-dependent tumor cell function, acting as a molecular switch that alters the balance between Rac1 and Rho activation, thereby skewing cell function toward a pro-migration (RAC1-dependent) or pro-TGF-β1 activation (Rho-dependent) phenotype.

MMP-9 is the main collagenase in keratinocyte, and it is required for many biological processes, such as wound healing.[92] It can digest the cell surface protein of the extracellular domain structure, thereby reducing the number of cell adhesions and increasing cell motility.[93] Recent studies have established a link between MMP-9 and tumor invasion, metastasis, and angiogenesis.[94] Yang[95] observed high αvβ6 and MMP-9 reactively in invasive tumors at 73.7% and 76.5%, respectively. αvβ6 expression and MMP-9 secretion are enhanced in high cell densities. Since integrin β6 only forms a heterodimer with integrin αv, detecting the mRNA or protein β6 subunit will provide dimer information.[96] Li[70] demonstrated that integrin β6 promotes tumor migration and invasion by activating Rac1 and upregulating MMP-9 expression in bile duct cancer cells, thereby establishing the integrin β6/Rac1/MMP-9 pathway.

According to these findings, integrin αvβ6 can promote tumor cell migration via the Eps8 and MMP-9-involving Rac1 pathway.

Integrin αvβ6 and ERK2

Mitogen-activated protein kinase cascades, which include ERK, JNK and P38, are critical intracellular signaling pathways that control a wide range of cellular functions.[97] Ahmed[56] demonstrated that the cytoplasmic domain of integrin β6 directly binds to ERK2, thereby increasing MAP kinase activity. According to Li,[69] integrin β6 significantly promotes pancreatic cancer cell proliferation and invasion and induces ETS1 phosphorylation in an ERK-dependent manner, resulting in MMP-9 upregulation. The absence of ERK2 binding sites in the cytoplasmic domain of integrin β6 affects density-dependent expression of integrin β6 and inhibits integrin β6-mediated MMP-9 secretion, thereby inhibiting tumor growth.[98] It is well established that the MAP kinase pathway promotes cancer growth in vivo. It plays a vital role in tumor metastasis, and integrin αvβ6 promotes MAP kinase pathway

activation.[99] Song[100] inhibited integrin αvβ6-mediated extracellular matrix degradation by inhibiting ERK activation, disrupted integrin αvβ6 internalization, and reduced cancer cell migration to fibronectin.[101] Additionally, Li[69] demonstrated that silencing integrin αvβ6 with small interfering RNA significantly inhibited the growth of pancreatic xenograft tumors in vivo, possibly via the ERK2 pathway.

EMT is considered a crucial process in tumor progression[102] and is closely associated with the systemic invasiveness of pancreatic tumors.[103] In non-Smad signaling responses, TGF-β activates the ERK–MAPK and other pathways to induce EMT.[102,104,105] Meanwhile, the ERK/MAPK signaling pathway is essential for chemical and immune resistance in tumor cells.[106] CCA and PDAC are resistant to chemo- and radiotherapy and are prone to metastasis. Targeting integrin αvβ6 on ERK2 may provide a novel treatment option for CCA and PDAC.

Integrinβ6 and PODXL

PODXL2, a newly discovered member of the CD34 family, is a type I transmembrane sialic acid protein that functions as an L-selectin ligand.[107] The role of PODXL2 in cancer is unclear, and its regulatory pathway has not been identified. PODXL1, an additional member of the CD34 family member, is overexpressed in several cancers, including pancreatic cancer.[108,109] It promotes cancer cell invasion by activating EMT in PDAC cells via the C5aR/C5a axis.[110] According to Soejima,[111] integrin β6 gene ITGB6 knock out significantly downregulates PODXL2 expression, which is significantly correlated with integrin β6 expression in CCA. Decreased PODXL2 expression inhibits the formation of intrahepatic cholangiocarcinoma (ICC) cells. It is further suggested that PODXL2 may be necessary to maintain the clonogenic potential of bile duct cancer cells. Currently, the relationship between integrin and PODXL is unknown. Studying the role of PODXL in the integrin β6 pathway may be a potential targeted therapy for CCA and PDAC (Figure 1).

Application of Integrin αvβ6 in Diagnosis, Treatment, and Prognosis of CCA and PDAC

Integrin αvβ6 as a Biomarker

As early as 2004, Sipos[112] studied gastrointestinal pancreatic cancer and found that all these cancer types overexpressed integrin αvβ6. The strongest expression was observed in PDAC and was not expressed in HCC and pancreatic neuroendocrine tumors. In gastrointestinal pancreatic cancer, integrin αvβ6 expression is limited to tumor cells. Steiger[113] conducted integrin αvβ6 immunohistochemical analysis on a large number of PDAC samples (383 primary tumors, 7 lymph nodes, 8 distant metastases, and 34 pancreatic intraepithelial neoplasia [PanIN]). The study found that integrin αvβ6 is highly expressed in primary PDAC foci (88%), metastatic foci (~100%), and PanIN (57%). According to Patsenker et al,[50] integrin αvβ6 is largely expressed in hilar cholangiocarcinoma (HCCA, 87%) and ICC (88%), with similar expression intensity in the two types of CCA. In contrast, integrin αvβ6 expression has been observed in HCC. Integrin αvβ6 immunohistochemical analysis distinguishes CCA and HCC with high specificity. This contradicts Franken's[114] finding that HCCA expresses αvβ6 more significantly than ICC; but, the difference in integrin αvβ6 expression between HCCA and ICC was too low to distinguish. Soejima[115] discovered that integrin αvβ6 was highly expressed in majority of the CCA cells, including ICC, and αvβ6 expression was higher in non-peripheral and periductal infiltration or intraductal growth types compared to peripheral and mass formation types. Integrin αvβ6 expression is closely related to ICC subtypes.

In terms of traditional imaging modalities, HCC and ICC overlap and are hard to distinguish; however, their treatment options and prognoses are different. Primary HCC treatment is liver transplantation, which is generally contraindicated in patients with ICC.[116] The distinction between HCC and HCCA is critical for patient treatment selection and prognosis. Integrin αvβ6 may be a molecular marker of epithelial malignancy. However, different results were reported in the above studies, providing a direction for future research. The following questions should be addressed. What is the reason for the difference between the different experiments? What is the relationship between the expression quantity in HCC, HCCA, and ICC?

Integrin αvβ6 as Imaging Point in Vivo

Carpenter et al used 4-[18F]fluorobenzoyl A20FMDV2 to quantify integrin αvβ6[117] in positron emission tomography (PET); however, the drug's low tumor uptake rate and metabolic instability limit its application. Nakamoto[118] developed a PET radiopharmaceutical preparation 18F-FP-R01-Mg-F2, which showed rapid, distinct, and specific uptake of tumors in cell and animal models. Localized lymph node metastases can be detected retroperitoneally, as can distant metastases in organs with low physiological intake, such as the lungs and liver. Hausner created a novel tracer by combining polydiethyl glycogen (PEG) and 18F-A20FMDV2,[119] and increased tumor uptake was observed. In addition, Hausner created a copper strain-free tracer [18F] FBA-C6-adibon3-PEg7-A20FMDV2, its uptake was observed in the gallbladder and gastrointestinal tract in animal studies.[120] This is undoubtedly a research direction for the treatment development of CCA lesions Further research would need to prove how the contrast agent binds CCA cells and exploit the mechanism, thus reducing the occurrence of false-positive development. In addition, researchers would need to understand how to noninvasively enrich the contrast agent in the bile duct and ensure that the body eliminates the agent in a specific time to reduce the occurrence of false-negative development.

Kimura additionally designed several highly stable cysteine binding peptides with high integrin αvβ6 affinity, that were labeled with 64Cu[121] or 18F.[122] Copper-labeled peptide 64Cu-NoTA-S02 showed promising in vivo imaging

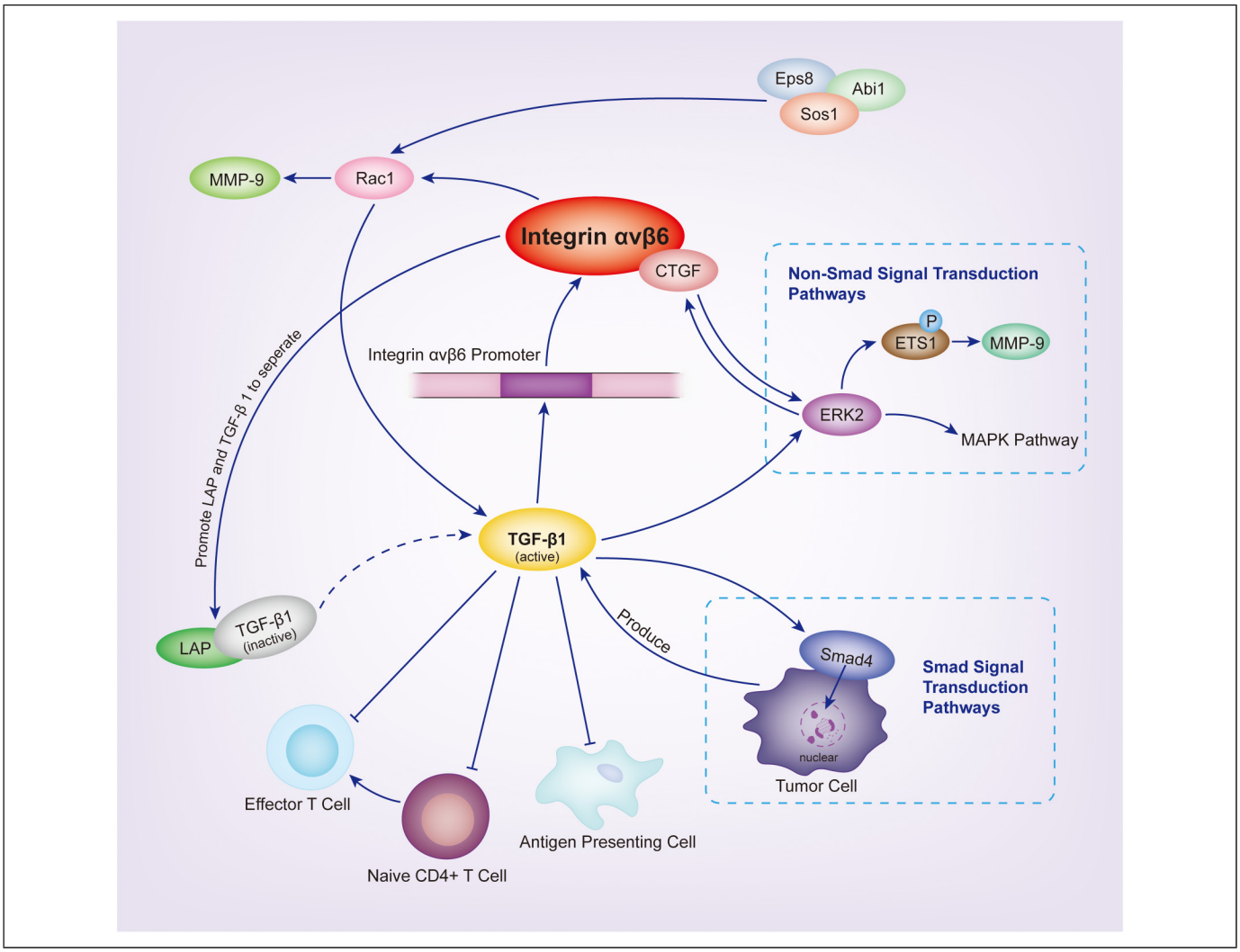

Figure 1. Interaction between integrin αvβ6 and the TGF-β1, ERK2, RAC1 signaling pathways.

properties. 18F allows for quick peptide elimination in vivo and has improved dynamic characteristics. Since linear peptides are rapidly metabolized in vivo, their clinical potential is limited.[123] At present, cyclized peptides have been proposed as a practical modification approach for improving peptide stability in vivo.[124] XunFeng et al created a novel 68Ga labeled cyclic peptide that targets the RGDLATL sequence based on integrin αvβ6. The imaging agent is simple to prepare and has good pharmacokinetic and dosiological characteristics; but, it has a low tumor uptake rate.[125]

In addition, Tummers[126] used IRDye800CW in conjunction with cysteine peptide R01-MG to guide the intraoperative near-infrared fluorescence imaging (NIRF) of PDAC. Liu[127] designed a new molecular probe, cy7.5-RGD /MSNs, to bind CCA tissue and label it fluorescently with ICG. These specialized imaging materials are critical for determining the intraoperative resection range of cancer focal margin and cancer treatment. Gao[128] synthesized a near-infrared phthalocyanine dye marker, dye-SA-B-HK, that specifically binds to integrin αvβ6 by streptavidin biotin chemistry, and once bound, cells can be viewed using optical imaging techniques. This can guide the anatomic location and complete surgical removal of in-situ pancreatic cancer lesions. Furthermore, under certain wavelength light irradiation, Dye-SA-B-HK demonstrated clear antitumor effects in vitro and in vivo. This suggests that we can create multifunctional molecular imaging agents with radioisotope and near-infrared phthalocyanine dye dual-labeling. This imaging agent enables the detection of primary and metastatic tumor foci using whole-body PET or SPECT scans, followed by optically image-guided tumor surgery and tumor-targeted phototherapy (PT).

At present, contrast agent research allows for non-invasive and accurate localization of PDAC. However, only PET and SPECT have been developed,[94,123] and contrast agents for ultrasound, x-ray and other imaging approaches have not yet been developed. The application of targeted integrin αvβ6 in optical imaging and localization of CCA is also rare. Several studies have shown that integrin αvβ6 is upregulated in ICC

and HCCA, but not in HCC.[50,70,129] Therefore, PET tracers can be used to differentiate cholangiocarcinoma from benign disease and cholangiocarcinoma from HCC prior to surgery, which is extremely important for improving treatment accuracy. Based on the tissue and embryo homology of CCA and PDAC, applying the PDAC imaging technology to CCA should be further investigated.

Integrin αvβ6 as a Therapeutic Target

It has been reported[130] that the ITGB6 gene is required for Ras oncogene-dependent PDAC cell growth, allowing tumor cell proliferation, migration, and invasion. Integrin αvβ6 blocking antibody therapy can inhibit tumor growth in animal models, suggesting integrin αvβ6 inhibition may serve as a potential cancer therapy.[130] Integrin αvβ6 is a novel therapeutic target for CCA and PDAC, due to its unique expression and essential role in epithelial tumors. A significant risk factor for CCA is biliary fibrosis. By demonstrating that integrin αvβ6 was upregulated in biliary fibrosis, Wang[49] showed that integrin αvβ6 plays a role in fibrosis response, and integrin αvβ6 or TGF-β inhibition prevents the development of biliary fibrosis. Liang[131] constructed integrin αvβ6-targeted immune liposomes and discovered that they could effectively inhibit tumor growth and induce apoptosis of cancer cells with tumor specificity and targeting. Soejima[111] knocked out the integrin β6 gene, *ITGB6*, in two CCA cell lines (with significantly high and low integrin αvβ6 expression). The results showed that, in both cell lines, cell migration, invasion, wound healing, colony formation, PODXL2 expression, and healthy cell cycle decreased. These results suggest that integrin β6 could serve as a therapeutic target or diagnostic marker. Eberlein[132] found that the therapeutic antibody, 264RAD, can target integrin αvβ6 activity and exert antitumor effects by decreasing expression in a dose-dependent manner, as well as significantly inhibit p-ERK in cancer cells. 264RAD delays tumor cell invasion and diffusion by inhibiting TGF-β activation and MMP-9 secretion. E-cadherin is overexpressed in regions of decreased avβ6 expression, suggesting that integrin αvβ6 effectively regulates different cancer pathways. Reader[130] applied 264RAD to PDAC animal studies. Tumors in KDC mice treated with 264RAD had significantly decreased proliferation ability (Ki67), tumor growth signal (pERK), vascular density (Endomucin), and TGF-β signal (nuclear Smad4), as well as decreased the expression of phosphorylated Smad3 and αSMA, and collagen deposition.

Since the discovery of the highly targeted integrin αvβ6 capsid protein A20FMDV2 in 2007,[133] many studies have been conducted on the efficient and low toxic transport of the capsid in foot-and-mouth disease. Tumor clearance was achieved by binding tesirine to A20FMDV2, which was selectively delivered to mouse pancreatic cancer foci.[134] Adenovirus 5 (Ad5) is a common tool in oncolytic virus therapy, and targeted therapy with αvβ6 integrin-binding peptide (A20) modified adenovirus AD5-3 δ-A20 T eliminates the off-target effect of Ad5 alone, in the liver and spleen, and significantly reduces pancreatic cancer progression.[135] CAR-T cell therapy is one of the most popular immunotherapies for hematological malignancies; but, it has not yet been approved for clinical use in solid tumors. Since IL-8 is overexpressed in TME, Whilding[136] injected mice with A20FMDV2-CXCR2-CAR cells, co-expressed by A20FMDV2 and the IL-8 receptor, which increased the number of T cells migrating to TME and improved the antitumor activity and tumor toxicity of CAR therapy. In addition, the tumor load was significantly reduced in mice.[136,137] Despite the fact that there are many targeted studies of integrin αvβ6 using A20FMDV2, there are no clinical studies to support these therapies, including the popular CAR-T therapies. The high affinity of A20FMDV2 to integrin αvβ6 appears to be a breakthrough indication for integrin αvβ6-targeted therapy. However, several research questions need to be addressed. How can these therapies be made safe and efficient? Can these technologies be applied clinically for the treatment of bile duct cancer and PDAC?

Hezel[138] blocked TGF-β1 and integrin αvβ6 expression in KRASPDAC mouse models and found that it accelerated disease progression in Smad4-expressing tumors. The tumor inhibition function of integrin αvβ6 was lost in tumors with homozygomorphic deletion of Smad4. This suggests that blocking TGF-β1 or integrin αvβ6 can reduce tumor cell migration but increase proliferation, accelerating disease progression. Targeting integrin αvβ6 may not always have an inhibitory effect on all tumors, and widespread use of TGF-β1 inhibitors in an unselected population of patients with PDAC may have negative consequences, especially in cancers with intact TGF-β/SMAD4 signaling pathways, and may accelerate disease progression. It is necessary and urgent to conduct in-depth and diverse studies on the integrin αvβ6 pathway in CCA and PDAC in order to identify downstream genes for more precise targeting, as well as to avoid unwanted tumor therapy results.

Moreover, epithelial-derived malignancies encompass more than just CCA and PDAC. Chernaya[139] found that integrin αvβ6 expression was up to 18-fold higher in papillary thyroid carcinoma when compared to that in normal tissues. This suggests that integrin αvβ6 is not limited to cholangiocarcinoma and may have similar implications for other epithelial cancers. As a result, more research is required to ascertain whether there are more precise markers of integrin αvβ6 in the CCA pathway (Table 1).

Integrin αvβ6 Combined with Drugs

Statins, also known as hydroxy-3-methylglutaryl (HMG-CoA) reductase inhibitors, are commonly used as lipid-lowering agents to inhibit cholesterol biosynthesis.[149] In addition to lowering blood lipids, statins are involved in a variety of physiological processes. In cancer, simvastatin inhibits PC3 micrometastasis in prostate cancer by inhibiting integrin αvβ3 activity,[150] and it also reduces tumor cell adhesion in human peritoneal mesenchymal cells by decreasing vCAM-1 and integrin β1 expression.[151] Studies on lovastatin have shown

Table 1. Studies on Integrin αvβ6 as Imaging Point and Therapeutic Target.

Peptide/drug	Pharmaceutical	Binding materials	Characteristics summary	References
A20FMDV2	[^{18}F]FBA-PEG28-A20FMDV2; [^{18}F]FBA-(PEG28)$_2$-A20FMDV2; [^{18}F]-FBAA20FMDV2-PEG28; [^{18}F]-FBA-PEG28-A20FMDV2-PEG28	^{18}F ; PEG 28	PEG increases biological half-life, metabolic stability, and tumor absorption. When compared to just one PEG, the addition of two PEGs inhibited drug clearance. Experiments show that adding one PEG 28 to the N-terminal and one PEG 28 to the C-terminal produces optimal results.	119, 140
	Ad-3Δ-A20T	Oncolytic adenovirus AdΔΔ	Ad5-3d-a20t retains the replication function of the virus, even in the presence of gemcitabine. It can effectively kill cancer cells, inhibit tumor growth, and weaken the ability of cancer cells to bind with red blood cells and blood factors. It also has a high binding affinity for PDAC.	141
	Ad-3Δ-A20T	^{125}I ; oncolytic adenovirus AdΔΔ	The addition of ^{125}I does not necessitate gene modification or significantly reduce viral biological activity. It directly radioactively labels the virus. ^{125}I shows biological distribution, elimination rate, and off-target effect rate of the adenovirus.	135
	PDC SG3299	Tesirine	SG3299 can cure PDAC with an optimal dosage and dosing regimen. The presence of pancreatic stellate cells has no effect on SG3299 cytotoxicity. SG3299 significantly reduces the spherogenesis of PDACPDX cells.	134
Knottins (Cystine forms peptides)[121]	^{99}m Tc-SAAC-S02	^{99}mTc	Rapid tumor targeting with renal clearance. High blood and liver uptake and retention rates. Low miss rate.	142
	^{18}F-FP-R01 and ^{18}F-FP-S02	^{18}F	^{18}F-FP-R01 intake was low in non-target tissues and healthy tissues, and moderate in the kidney. ^{18}F-FP-R01 outperforms ^{18}F-FP-S02 in terms of tumor affinity and plasma stability.	122
	R01-MG-IRDye800	IRDye800	Tumor-specific targeting and a high rate of renal clearance. High tumor background ratio and a clear correlation were observed between fluorescence signal and the PDAC histopathology.	126
	^{18}F-FP-R01-MG-F2	^{18}F	Tumor accumulation is faster, higher, and stable. Uptake of ^{18}F-FP-R01-Mg-F2 in retroperitoneal lymph node metastasis was reported. Distant metastases are visible in organs with low physiological intake, such as the lungs and liver. Knottin may be able to meet the need for accurate cancer spread assessment.	143, 118
	^{177}Lu-DOTA-integrin αvβ6 knottin	^{177}Lu	It is a high-affinity tracer for PDAC with high tumor accumulation and moderate, rapidly declining renal uptake.	96
HK (TP H2009.1)	^{99}m Tc-HHK	^{99}m Tc	With rapid tumor accumulation, the radiotracer showed maximum tumor uptake at 0.5h after injection. The tumor can be seen with high contrast but it has a low uptake rate. It has high sensitivity and accuracy in identifying and localizing small metastatic liver lesions.	123
	Dye-SA-B-HK	IRDye700	Dye-SA-B-HK has a high receptor-binding affinity, indicating that dye-SA-B-HK could be enriched in tumors in large and specific quantities. It can be used as photodynamic therapy (PDT) to necrotize cancer cells and reduce tumor proliferation.	128
	^{99}m Tc-HYNIC-cHK	^{99}m Tc	In vivo, cyclic HK peptide (cHK) had similar biological distribution characteristics to linear HK peptide but significantly improved metabolic stability and rapid tumor accumulation in vivo. The	144

(continued)

Table 1. (continued)

Peptide/drug	Pharmaceutical	Binding materials	Characteristics summary	References
			binding affinity of cHK to integrin αvβ6 was slightly lower than compared to that of the HK peptide, which might be attributed to peptide sequence shortening and conformational constraints.	
Cycratide	^{68}Ga-cycratide	^{68}Ga	It is easy to prepare and has good pharmacokinetic and biological characteristics. Cleared by renal and bladder pathways, the blood and surrounding abdominal organs have a low background, it can be sensitively detected in pancreatic neoplasms, and is internally stable.	125
IsoDGR	^{99}mTc-3PisoDGR	^{99}m Tc	IsoDGR has the ability to target both αvβ6 and α5β1. This strategy avoids the tedious labor and timely preparation. It avoids the interaction of two different targeted molecules. High tumor uptake.	145
αvβ6-BP	[^{64}Cu]Cu DOTA-EB-αvβ6-BP([^{64}Cu]1) and [^{64}Cu]Cu DOTA-IP-αvβ6-BP([^{64}Cu] 2)	^{64}Cu ; Albumin binding moirty (ABM) (EB、IP)	Rapid tumor uptake, increased circulation time, decreased renal uptake, rapid clearance, and increased tumor accumulation. [^{64}Cu]2 is stable in serum and improves tumor visualization.	146
Avebehexin	Ga-68-avebehexin	^{68}Ga	Polymerization has no effect on the αvβ6 integrin-selective peptide C (FRGDLAFp(NMe)K), and the TRAP-conjugated monomer ^{68}Ga-Avebehexin can achieve highly sensitive PET imaging as well as therapeutic effects. Despite the fact that tumor intake is low, it is still higher than in all other organs, except the kidney.	147
Trivehexin	Ga-68-Trivehexin	^{68}Ga	^{68}Ga-trivehexin showed significant selectivity to integrin αvβ6 over other RGD-binding integrins. With the exception of the kidney, nonspecific uptake is almost completely eliminated. More specific and sensitive metastatic cancer diagnosis.	148

that it induces cytoskeletal changes and, as a result, regulates adhesion, motility, and proteolysis.[151]

Recent studies have focused on the use of statins to treat CCA.[149,152,153] Simvastatin inhibits cholangiocarcinoma proliferation by inhibiting Rac1 activity[154] or downregulating E2F-1/TS.[153,154] Yang et al[155,156] showed that lovastatin inhibits ICC proliferation and aids in the treatment of gefitinib-resistant CCA. In general, statins may have potential proliferative inhibitory and therapeutic effects on cholangiocarcinoma via interaction with the integrin pathway.

Gemcitabine is used as a baseline treatment for PDAC and CCA; however, due to its treatability and chemotherapeutic resistance, gemcitabine is ineffective when used alone.[157] Based on this fact and the poor response to ICIS treatment, there is no more effective treatment for PDAC patients. It is urgent to continue studying the drug mechanism and developing more effective projects to overcome drug resistance.[158] Reader[130] administered a combination of gemcitabine and 264RAD (an integrin αvβ6 inhibitor) to tumor-bearing mice. The combination treatment resulted in significantly smaller or even complete tumor disappearance. However, the specific mechanism has yet to be clarified. Studies[159] have focused on explaining the mechanism by which silencing ADP Ribosylation Factor 6 (ARF6) reduces gemcitabine resistance in pancreatic cancer. ARF6 is located downstream of the Kras/Erk signaling pathway and has been shown to induce lipid peroxidation. Because reactive oxygen species (ROS) can promote gemcitabine resistance in pancreatic cancer,[160] inhibiting ARF6 can improve gemcitabine sensitivity. The 264D antibody can block pErk growth signals. Therefore, the integrin αvβ6 inhibitor may increase the sensitivity of gemcitabine via the Erk pathway. Although integrin αvβ6 has been shown to be part of an effective cancer treatment, it has also been demonstrated that integrin αvβ6 participates in HCC resistance to cisplatin via ERK/MAPK signaling.[161] However, due to the promising results of Reader's trial, if we continue to further study the mechanism and combined application of integrin αvβ6 and various chemotherapeutic drugs or other targeted drugs, we may be able to solve the problem of poor treatment effect caused by PDAC resistance to chemotherapeutic drugs and translate the research result into clinical application to bring good news to PDAC patients.

Integrin αvβ6 as a Prognostic Factor

Integrin αvβ6 is known to promote epithelial cancer cell proliferation, migration, and invasion via multiple pathways. Through experiments and data analysis, Li[70] discovered that integrin β6 increased Rac1-GTPase, resulting in MMP-9 upregulation and F-actin polymerization, which promoted tumor invasion. The study results concluded that increased integrin β6 inhibition in CCA is related to lymph node metastasis and distant metastasis. Through extensive statistical analyses on HCCA, Sun[161] discovered that integrin αvβ6 expression is significantly correlated with tumor differentiation and lymph node metastasis. Reader[130] examined 491 PDAC cases and found that the survival rate of patients with PDAC correlates with the expression of integrin αvβ6; the higher the integrin αvβ6 expression, the lower the survival rate of patients.

When cancer metastasizes, the prognosis of patients is often poor. Reader[130] detected the expression level of integrin αvβ6 in six patients with primary PDAC tumor tissues with corresponding lung, colon, or liver metastases, and concluded that integrin αvβ6 was strongly expressed in both primary and metastatic sites. In addition, the expression intensity in 70% of the metastatic samples was similar to that of the primary tumor, suggesting that non-invasive screening of cancer metastasis can be accomplished using appropriate imaging methods. It provides us with an additional prognosis method, even though there is still a false-negative rate.

Integrin αvβ6 is a potential biomarker for cancer diagnosis and tumor metastasis detection. In the future, the range of integrin αvβ6 expression corresponding to different grades of CCA and PDAC can be obtained by sequencing analysis of a large number of clinical cases, guiding clinical diagnosis and prognosis.

Discussion

CCA and PDAC are difficult to diagnose and treat, and have extremely low survival rates. Therefore, advances are required to improve cancer diagnosis, treatment, and prognosis. Integrin αvβ6 is specific to epithelial malignant tumors, such as CCA and PDAC, which share the same histopathological origin and similar manifestations. It plays a vital role in the occurrence, development, and proliferation of cancer. The higher the integrin αvβ6 expression, the worse the cancer prognosis. These results suggest that regulating integrin αvβ6 or its upstream and downstream genes may help to reduce the progression of cancer. The development of molecular imaging agents that work in conjunction with integrin αvβ6 can allow for effective visualization of anatomic location, thus guiding diagnosis and surgery. In addition, integrin αvβ6 inhibitors can be used in combination with Gemcitabine and other drugs to enhance cancer treatment. The expression level of integrin αvβ6 can also influence prognosis.

In addition, integrin αvβ6 expression is increased in a variety of fibrotic diseases, including idiopathic pulmonary, liver, and renal fibrosis; limiting its specificity as a prognostic marker.[143] However, the properties of integrin αvβ6 can be exploited for imaging, through imaging technology such as PET, to evaluate its expression (and possible cancer stage). It can be integrated with the expression levels of other molecular markers for a more comprehensive assessment, precisely estimating cancer prognosis and excluding other disease effects. In addition, bile duct fibrosis can lead to carcinogenesis, and studies have shown that integrin αvβ6 inactivation effectively inhibits ductal reaction in vivo, inhibiting the progression of biliary fibrosis and tumorigenesis. Therefore, the use of targeted agents may be beneficial for cancer prevention.[162]

Furthermore, several questions remain unsolved, including the integrin αvβ6 mutation rate, the impact of other gene

mutations on integrin αvβ6 expression, and the efficacy of targeted agents when other gene mutations or integrin αvβ6 mutation occur. Understanding these associations may aid in the identification of high-risk patient populations and the development of individualized treatment methods for patients with these specific gene mutations. Further exploration is still needed to address the remaining questions.

Further exploration into the relationship between integrin αvβ6 and cancer, and additional drugs like lovastatin and gemcitabine that act on integrin αvβ6, as well as adjuvant imaging, and targeted therapy molecules like A20FMDV2, are important directions for treating CCA and PDAC with integrin αvβ6. Further research is also needed to determine how to avoid tumor proliferation when TGF-β and integrin αvβ6 are blocked in homozygous Smad tumors.

Various studies on the effective CCA and PDAC diagnosis and treatment are ongoing; however, the mechanisms are still not fully clarified. Studies on combination therapy with currently recommended methods have not been reported; however, since integrin αvβ6 is now known to have promising expression and imaging characteristics, we believe that research in this area will increase continuously over the next five years. Finally, the advantages and drawbacks of integrin αvβ6 will be presented in greater detail.

This review has some limitations. Due to the article's unique starting point, which begins with two related cancers, and the focus of integrin αvβ6 research on these two cancers differs, parts of the article focus more on one cancer. In addition, due to the focus of this review on the molecular marker integrin αvβ6, other ongoing treatment studies for PDAC and CCA were only briefly reviewed. In addition, we have included only extensively studied imaging agents, but emerging and less extensively researched image agents are not be included.

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Ethical Approval

Not applicable, because this article does not contain any studies with human or animal subjects.

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