# Arginine metabolism: a potential target in pancreatic cancer therapy

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**Abstract**

Pancreatic ductal adenocarcinoma (PDAC) is an extremely malignant disease, which has an extremely low survival rate of <9% in the United States. As a new hallmark of cancer, metabolism reprogramming exerts crucial impacts on PDAC development and progression. Notably, arginine metabolism is altered in PDAC cells and participates in vital signaling pathways. In addition, arginine and its metabolites including polyamine, creatine, agmatine, and nitric oxide regulate the proliferation, growth, autophagy, apoptosis, and metastasis of cancer cells. Due to the loss of argininosuccinate synthetase 1 (ASS1) expression, the key enzyme in arginine biosynthesis, arginine deprivation is regarded as a potential strategy for PDAC therapy. However, drug resistance develops during arginine depletion treatment, along with the re-expression of ASS1, metabolic dysfunction, and the appearance of anti-drug antibody. Additionally, arginase 1 exerts crucial roles in myeloid-derived suppressor cells, indicating its potential targeting by cancer immunotherapy. In this review, we introduce arginine metabolism and its impacts on PDAC cells. Also, we discuss the role of arginine metabolism in arginine deprivation therapy and immunotherapy for cancer.

**Keywords:** Pancreatic cancer; Arginine deiminase; Arginine metabolism; Therapy

## Introduction

Pancreatic cancer is a highly malignant tumor of the digestive system that has high rates of metastases and poor survival rates. Pancreatic ductal adenocarcinoma (PDAC) accounts for >85% of all malignant pancreatic exocrine tumors and is the fourth leading cause of cancer-related death in both males and females with a 5-year survival rate of <9% in the US population.[1] Due to the lack of the early typical symptoms and extremely aggressive characteristics, >80% of patients with PDAC miss their chance of surgery upon diagnosis.[2]

In contrast to the patients with resectable diseases, who show a considerable response to adjuvant treatment with FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin), patients with advanced-stage PDAC only exhibit modest improvement in overall survival.[3] Although *KRAS* mutations have been detected in 95% of PDAC, many attempts to clinically target mutated *KRAS* have been unsuccessful.[4] In addition, immunotherapy, such as immune checkpoint inhibitors, also showed disappointing results in clinical trials of patients with advanced-stage PDAC, due to the lower tumor mutational burden and the dense desmoplastic stroma.[5,6] The effective application of chimeric antigen receptor T-cell (CAR-T) immunotherapy in patients with solid tumors, such as pancreatic cancer, remains a challenge due to the complex tumor microenvironment, the stromal hindrance in limiting immune response, and the expression of molecules associated with the immune checkpoint blockade.[7] Therefore, alternative therapeutic strategies for patients with advanced or metastatic pancreatic cancer are urgently needed.

Novel therapeutic strategies that target tumor metabolism are increasingly intriguing and are expected to contribute to the future management of patients with PDAC. Screening and validation of key metabolic enzymes, in the significantly reprogrammed metabolic pathway, have been used as methods to discover potential therapeutic targets.[8-10] To overcome drug resistance, researchers attempted to develop drugs that could simultaneously inhibit various enzymes associated with certain metabolic pathways. For instance, Leone *et al*[11] demonstrated that the treatment of tumor-bearing mice with the glutamine antagonist 6-diazo-5-oxo-L-norleucine suppressed pancreatic cancer metabolic programs and enhanced antitumor immune response. This effect was associated with the conditioning of $CD8^+$ tumor-infiltrating lymphocytes toward a highly proliferative, active, and long-lived

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phenotype that could replenish the tricarboxylic acid cycle intermediates by upregulating glucose anaplerosis.

Therapies that target arginine metabolism have attracted increasing attention.[12-14] Arginine, a non-essential or semi-essential amino acid, is involved in various biological functions, such as cell proliferation, survival, and protein synthesis. It also serves as a precursor associated with the production of nitric oxide (NO), polyamines, prolines, creatine, and glutamate.[15] Notably, arginine has connections to metabolic pathways that are important for tumorigenesis and tumor progression, including NO, creatine, and polyamine synthesis pathways.[16-18] Argininosuccinate synthetase 1 (ASS1) is the key enzyme during arginine biosynthesis and various types of cancer cells that do not express this enzyme, such as human melanoma and hepatocellular carcinoma (HCC) cells, can be sensitive to arginine deprivation treatment, such as arginine deiminase (ADI). In this review, we aim to discuss the role of the arginine metabolism in PDAC and its potential application in pancreatic cancer targeted therapies.

## Arginine Synthesis and Degradation

In the adult human body, arginine can convert from a non-essential into an essential amino acid under some physiological stress conditions, such as burns, injury, and small intestine and kidney damages.[19] Citrulline is the precursor of arginine formation and endogenous arginine can be synthesized in the kidneys and liver through the essential involvement of ASS1; however, there is no net production of arginine in the liver due to the abundance of arginase (ARG) that can immediately catalyze arginine to generate urea and ornithine, which reduces blood ammonia, a metabolic pathway known as the ornithine cycle.

Cationic amino acid transporter (CAT) proteins are the major vehicles for arginine transport from the extracellular environment. There are four types of enzymes that degrade arginine, including ARG, which is the most essential determinant enzyme, nitric oxide synthetase (NOS), arginine decarboxylase (ADC), and arginine: glycine amidinotransferase (AGAT), and the main generated products are ornithine, urea, NO, glutamate, polyamine, and proline.[20] ARG has two existing isoforms, ARG 1 and 2. The former is located in the cytoplasm and is mainly expressed in liver cells, where it participated in the urea cycle, while the latter is located in the mitochondria and has a very low expression in extrahepatic cells. NOS has three isoforms: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS), which catalyze the conversion of arginine into citrulline, produce NO, and play different roles in different organs.

## Arginine Metabolism in PDAC

Due to the enhanced metabolism, tumor cells take up a massive amount of nutrition compared with normal cells, and this process requires special transporters for organic materials. In ASS1-deficient cancer cells, the expression of arginine transporter gene, solute carrier family seven member two (*SLC7A2*) and family three member two (*SLC3A2*), is increased, indicating an enhanced reliance on arginine transport from the extracellular environment.[16] In pancreatic cancer tissue, it was reported that *SLC3A2* is highly expressed in 56.7% of cases.[21] Additionally, the expression of *SLC7A2* was notably associated with prognosis in patients with PDAC.[22] RNA-seq analysis and chromatin immunoprecipitation (ChIP) experiments identified *SLC7A3* as an important player which was found to be transcriptionally induced by p53 under glutamine deprivation. The increased intracellular arginine levels following glutamine deprivation are p53 dependent and serve as effectors for mammalian target of rapamycin complex 1 (mTORC1) activation that promotes cell proliferation and tumor growth [Figure 1].[23] In addition, the pancreatic cancer cells can non-selectively obtain multiple amino acids through micropinocytosis.[24]

ASS1 converts citrulline to argininosuccinate and is the rate-limiting enzyme in arginine synthesis. Its expression is generally high in normal tissues but lost in a range of tumor types. There have been various cancer types including melanoma, HCC, renal cell carcinoma, prostate cancer, osteosarcoma, and pancreatic cancer that have been identified as promising or potential targets for ASS expression-dependent arginine deprivation therapy.[12,13,25] Thus, arginine starvation that is mediated by ARG or ADI in ASS1-negative tumors has been considered as an available strategy of oncotherapy. The mechanism behind the expression loss of ASS1 is cancer type-dependent and is associated with the methylation of the ASS1 promoter that appears to mediate the repression of ASS1 in lymphoma, myxofibrosarcoma, nasopharyngeal carcinoma, HCC, and malignant pleural mesothelioma. In addition, the interplay between c-Myc and hypoxia-inducing factors-1 alpha (HIF-1α) controls ASS1 expression level in melanoma.[16] In pancreatic cancer, ASS mRNA and protein levels are altered in different cell lines. For instance, ASS1 expression is higher in BxPC3 cells compared to that in PANC1 cells.[26] Meanwhile, it was found that PANC1 has a higher level of ASS promoter methylation that was assessed using a detection method based on a methylation-specific polymerase chain reaction (PCR). According to the previous studies, the expression of ASS1 is low or negative in Panc1, AsPC1, and MiaPaca2, but high or positive in BxPC3 and Capan1.[14,27] Besides, ASS1 may also have a tumor suppressor function.[27-29] ASS1 low expression significantly correlated with high-grade pancreatic cancers and the poor prognosis of patients.[27] Additionally, it was shown that pancreatic cancers that have a reduced ASS expression were associated with a higher survivin expression, and increased lymph node metastasis and local invasion.[30]

ARG catabolizes arginine into ornithine and urea and its two types share approximately a 60% amino acid sequence similarity and a 100% homology in the regions that are vital to their enzymatic function.[31] Compared with ARG2, ARG1 is more stable and effective, and ARG1 is closely associated with M2 macrophages where it serves as a marker, while ARG2 can regulate M1 macrophages. In pancreatic cancer, researchers developed a real-time PCR-based assay to monitor the transcription levels of ARG1 and iNOS to assess the polarization status of tumor-induced

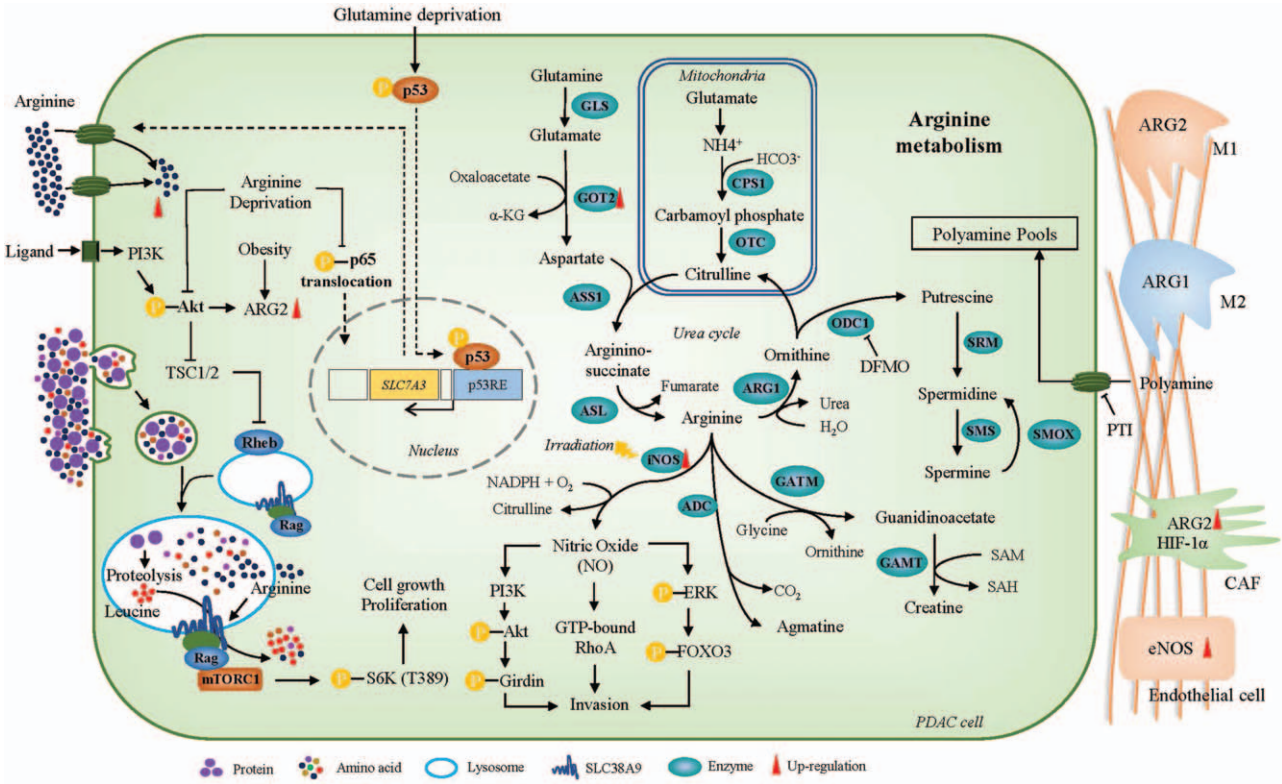


**Figure 1:** Arginine metabolism in PDAC. The PDAC cells can acquire arginine through transporters, micropinocytosis and intracellular synthesis. ARG1/2, iNOS, ADC, and GATM are the four main enzymes that degrade arginine. In addition, arginine metabolism participates in the regulation of signaling pathways. ADC: Arginine decarboxylase; Akt: Protein-serine-threonine kinase; α-KG: α-ketoglutarate; ARG1: Arginase 1; ARG2: Arginase 2; ASL: Arginine succinate lyase; ASS1: Argininosuccinate synthetase 1; CAF: Cancer-associated fibroblast; CPS1: Carbamoyl phosphate synthetase 1; DFMO: Difluoromethylornithine; eNOS: Endothelial nitric oxide synthetase; ERK: Extracellular regulated protein kinases; FOXO3: Forkhead box protein O3; GATM: L-arginine:glycine amidinotransferase; GAMT: Guanidinoacetate N-methyltransferase; GLS: Glutaminase; GOT2: Glutamic-oxaloacetic transaminase 2; GTP: Guanosine triphosphate; $HCO_3^-$: Bicarbonate ion; HIF-1α: Hypoxia-inducing factors-1 alpha; iNOS: Inducible nitric oxide synthetase; M1: M1 macrophage; M2: M2 macrophage; mTORC1: Mammalian target of rapamycin complex 1; NADPH: Nicotinamide adenine dinucleotide phosphate; $NH_4^+$: Ammonium; ODC1: Ornithine decarboxylase; OTC: Ornithine transcarbamylase; P: Phosphorylation; p53RE: p53 responsive element; PDAC: Pancreatic ductal adenocarcinoma; PI3K: Phosphatidylinositol-3-kinases; PTI: Polyamine transport inhibitor; SAH: S-adenosyl-L-homocysteine; SAM: S-adenosylmethionine; SLC3a2: Solute carrier family 3 member 2; SLC7a3: Solute carrier family 7 member 3; SMOX: Spermine oxidase; SMS: Spermine synthase; SRM: Spermidine synthase; TSC1: Tuberous sclerosis 1 protein; TSC2: Tuberous sclerosis 2 protein.

macrophages under hypoxic conditions.[32] In addition, researchers found that ARG2 expression was shown in α-smooth muscle actin positive cancer-associated fibroblasts (CAFs) by immunohistochemistry, especially those located within or around necrotic areas, through examining over 200 cases of PDAC, but it was rarely demonstrated in PDAC cells. Such ARG2-expressing CAFs co-expressed with HIF-1α and their presence in PDAC tissues was closely related to shorter overall survival of the pancreatic cancer patients.[33] In addition, Zaytouni *et al*[34] demonstrated the role of ARG2 in obesity-associated pancreatic tumors. The obesity-driven PDAC exhibited an accelerated growth and a transcriptional enrichment for pathways that regulate nitrogen metabolism and that involve glutamine, aspartate, and arginine metabolisms. The expression of ARG2 was upregulated upon obesity or AKT activation and its silencing or loss caused a significant accumulation of ammonia and suppression of PDAC. Notably, ARG1 but not ARG2 is the main hepato-ureogenesis enzyme in mammals,[35] therefore, specific targeting of ARG2 may provide a therapeutic opportunity for the treatment of PDAC patients who suffer from obesity and metabolic syndromes with less adverse effects.

Tumors, such as breast and colon cancers, with high expression of ARG exhibit rapid growth rates, due to the accumulation of polyamines.[36,37] The ornithine decarboxylase (ODC) is a key enzyme that catalyzes ornithine into putrescine during the polyamine metabolism. The ODC inhibitor, difluoromethylornithine (DFMO), was demonstrated to exert an anticancer effect.[38,39] To sustain their high growth rates, PDACs alter the polyamine metabolism that is reflected by the presence of high intracellular polyamine levels and their upregulated import of exogenous polyamines via organic cation transporters (OCTs). Researchers found that pancreatic cell lines have excess polyamine pools and their rebalancing may address the deficiency, induced by polyamine biosynthesis inhibitors that target metabolic enzymes, such as ODC, spermidine synthase, or spermine synthase (SMS). Notably, a combination therapy that contains an ODC inhibitor, such as DFMO, an SMS inhibitor, and a polyamine transport inhibitor (PTI), significantly depleted the intracellular polyamine pools and reduced PDAC cell growth.[40] Hence, we considered that targeting arginine metabolism is a potential and valuable therapeutic strategy.

NOS catalyzes the production of NO from L-arginine and it was reported that iNOS and eNOS are overexpressed in PDAC tissues compared with normal tissues and that high level of iNOS expression is associated with pancreatic cancer progression.[41-44] NO is an important cellular signaling molecule that participates in angiogenesis and due to its important role in cancer, the function of its precursor, L-arginine, on cancer growth and metastasis should be further studied.

## Arginine Participates in Signal Transduction

The mTORC1 protein kinase that is dysregulated in cancer regulates cell growth and metabolism.[45] Growth factors, energy, and amino acids jointly regulate the activation of mTORC1. Amino acids can promote mTORC1 interaction with the Rag GTPases that localize in lysosomes where Rheb directly stimulates mTORC1 kinase activity after stimulation by growth factors and energy. Chantranupong *et al*[46,47] found that mTORC1 could be activated through arginine sensing that is mediated by the cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1) and solute carrier family 38 member nine (SLC38A9). Arginine deprivation inhibits mTORC1 via CASTOR1 and GTPase-activating proteins toward Rags (GATOR2) interaction. CASTOR1 arginine-binding capacity is required for arginine to activate mTORC1 via disrupting the CASTOR1–GATOR2 complex.[46] Moreover, by serving as a lysosomal messenger, the intracellular arginine activates SLC38A9 and promotes its interaction with the Rag GTPase–Ragulator complex. SLC38A9, a lysosomal 11-transmembrane segment protein with homology to amino acid transporters, is needed to transport most essential amino acids, especially leucine, out of lysosomes, where mTORC1 senses them through the cytosolic Sestrin proteins.[47,48] Arginine regulates the lysosomal concentration of many essential amino acids and increases the level of phosphorylation of p70 S6 kinase 1 (p-S6K1). Many essential amino acids accumulate in lysosomes while lacking SLC38A9. Thus, arginine coupled with mTORC1 activation released essential amino acids from lysosomes, which facilitate the growth of PDAC cells that can obtain amounts of amino acids via the macropinocytosis of extracellular proteins.[49,50]

The induction of arginine deprivation by ADI can inhibit the activation of the nuclear factor kB (NF-kB) survival pathway by blocking NF-kB p65 signaling via suppressing the nuclear translocation and phosphorylation (serine 536) of NF-kB p65, which can be utilized to potentiate gemcitabine (GEM) antitumor effects on Panc-1 cells.[30] The target proteins that are regulated by the NF-kB pathway include cell cycle regulators, apoptotic factors, growth factors, adhesion molecules, and prometastatic and angiogenic factors. Thus, it is important to investigate the mechanism by which the arginine metabolism regulates NF-kB pathway to lay the foundation for the development of an antitumor therapy that is based on inhibiting the activity of NF-kB.

The phosphatidylinositol-3-kinases/protein kinase B (PI3K/AKT) pathway was also altered due to the decrease in Akt phosphorylation when pancreatic cancer cells underwent arginine deprivation.[26] As mentioned earlier, NOS catalyzes the production of NO from L-arginine. Lim *et al*[42] reported that the inhibition of eNOS phosphorylation inhibits tumor initiation and maintenance. In turn, eNOS activation by AKT enhances the nitrosylation and activation of the endogenous wild-type Ras protein, thus promoting tumor growth. Moreover, NO can promote the invasion of pancreatic cancer cells by activating the PI3K/AKT–Girdin, GTP-bound RhoA, and extracellular regulated protein kinases (ERK)–forkhead box protein O3 (FOXO3) pathways.[43,51] The iNOS deficiency in KPC mice resulted in improved survival and lower tumor severity.[43] Therefore, we consider that arginine plays an important role in pancreatic tumor progression via direct activation of mTORC1 or indirect regulation of NO-related signaling pathways. To confirm this hypothesis, further verification is required.

## The Role of Arginine Metabolism in PDAC Cells

ADI treatment of ASS1-deficient Panc-1 cells decreased their proliferation in a dose- and time-dependent manner [Figure 2].[30] As mentioned earlier, arginine can induce mTORC1 activation, which leads to essential amino acids release from lysosomes, and regulate the PI3K/AKT and NF-kB signaling pathways, which facilitates the growth of PDAC cells.[26,30,47] Furthermore, arginine deprivation could significantly upregulate the expression of asparagine synthetase (ASNS) which redirects aspartate from *de novo* nucleotide biosynthesis, leading to nucleotide insufficiency and the triggering of cell cycle arrest at the S phase.[27] Additionally, arginine catabolism is the only source of the production of intracellular NO which can regulate cancer cell proliferation and growth. For instance, a low concentration of NO could stimulate tumor growth, while a high concentration results in cytotoxicity associated with the induction of DNA damage and apoptosis.[52] Moreover, it was shown that cancer cells with wild-type p53 were more sensitive to cytotoxicity that was mediated by NO when compared to cancer cells with mutant p53.[53]

Amino acid deprivation has been known as an activator of autophagy. It was demonstrated that [HuArgI (co)-PEG5000]-induced arginine deprivation leads to autophagy-dependent cell death, instead of caspase-dependent apoptosis in pancreatic cancer cells, which results in G0/G1 cell cycle arrest in the surviving cell fraction.[12] PDAC cell lines, such as Capan-1, that express ASS1 were rescued by the addition of excess L-citrulline, which indicated a partial arginine auxotrophy. However, arginine deprivation was found to induce apoptotic cell death in earlier research and other malignancies, such as acute leukemia.[54,55] In addition, the contribution of autophagy to arginine deprivation-induced cell death remains controversial.

In recent years, many studies demonstrated the role of arginine in cancer metastasis. Wang *et al*[26] showed that arginine starvation by ADI inhibits the metastasis of pancreatic cancer cells by influencing the markers of epithelial-mesenchymal transition. As the only precursor available for the production of NO, arginine is believed to promote the invasion of pancreatic cancer cells via NO

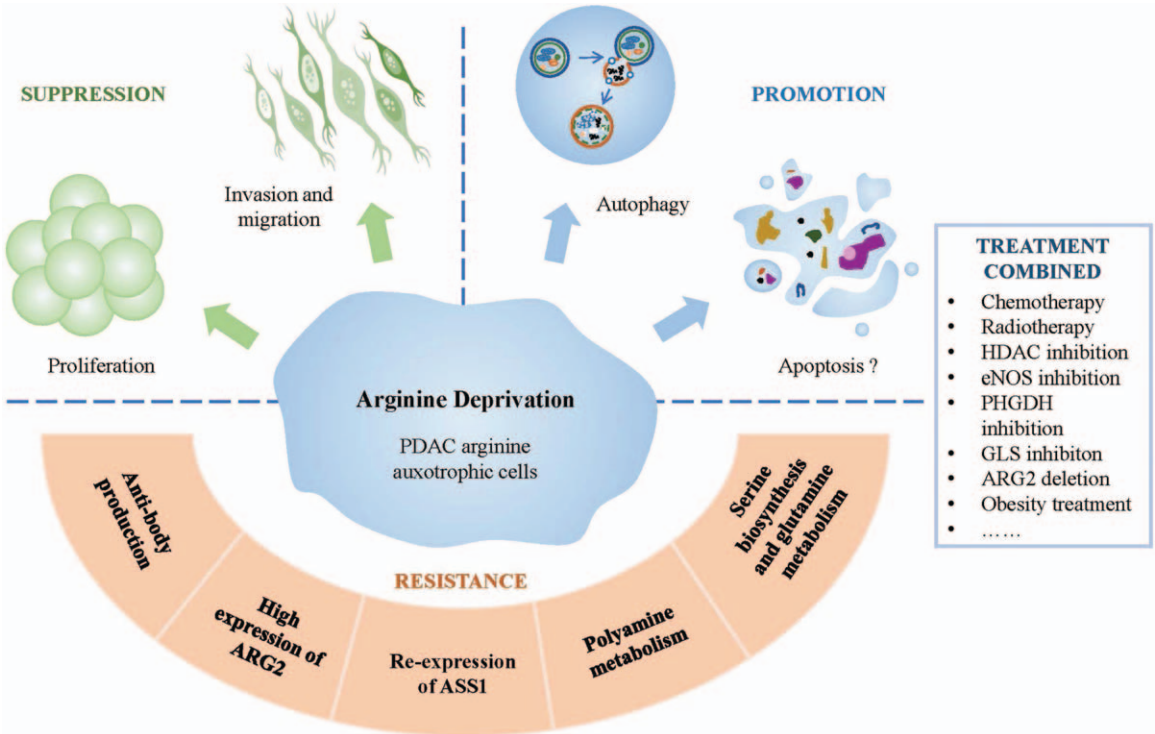


**Figure 2:** Arginine deprivation and resistance. Arginine deprivation inhibits the proliferation, invasion and migration of PDAC cells, and promotes autophagic death. The mechanisms of resistance to arginine deprivation involve the production of antibodies, high expression of ARG2, re-expression of ASS1 and altered intracellular metabolism. ARG2: Arginase 2; ASS1: Argininosuccinate synthetase 1; eNOS: Endothelial nitric oxide synthetase; GLS: Glutaminase; HDAC: Histone deacetylases; PDAC: Pancreatic ductal adenocarcinoma; PHGDH: Phosphoglycerate dehydrogenase.

regulatory mechanism, involving RhoA, PI3K/AKT, and ERK–FOXO3 signaling pathways.[43,51] Several studies reported on the relationship between NO and the vascular endothelial growth factor (VEGF) that could be upregulated by NO via the activation of HIF-1α, which promotes angiogenesis in various human cancers.[56] As for the NO synthetase, eNOS is overexpressed in the vasculature and peritumoral tissue of PDAC and is associated with the vascularization and neovascularization of pancreatic cancer.[57] NOS inhibition combined with VEGF receptor 2 blockade can significantly potentiate the anti-vascular therapeutic efficacy, leading to significant inhibition of pancreatic tumor growth compared to either therapy alone.[58] The results of these studies indicate the important role of arginine metabolism in PDAC progression and suggest that therapies that target arginine metabolism could be a potential strategy for the treatment of pancreatic cancer.

## Arginine Deprivation Therapy for PDAC

Certain tumors are unable to independently synthesize arginine, a process known as arginine auxotrophy. Therefore, arginine depletion that is induced by ARG and ADI shows a great efficiency when applied for the treatment of various arginine auxotrophic tumors.[20] As a non-essential amino acid, arginine can be slowly synthesized in the human body and arginine depletion treatment does not produce hyperammonemia or orotic aciduria, which indicates less adverse effects. ADI cannot be produced by mammals and is derived from microorganisms. Thus, researchers linked the drug with polyethylene glycol (PEG) to increase its circulating half-life and decrease its immunogenicity. Due to the different metabolic processes, the strategy of using the two enzymatic agents for cancer treatment varies. ADI–PEG was effective in the treatment of tumors with ASS1 deficiency, and the pegylated ARG might show an anticancer effect with either ASS1 or ornithine transcarbamylase (OTC) deficiency.

ASS1-deficient pancreatic cancer exhibits an apparent tumor growth inhibition under arginine deprivation that is induced by ADI or ARG treatment. Importantly, arginine auxotrophy sensitizes pancreatic cancer to chemotherapy, radiotherapy, and other therapies. There was a study that illustrated the mechanism by which arginine deprivation could potentiate the antitumor effect of GEM on Panc-1 cells via multiple mechanisms, including the induction of cell cycle arrest in the S phase, the upregulation of caspase-3 and 9 expression, and the inhibition of NF-kB pathway's activation.[30] In addition, studies reported that ADI-PEG20 could increase the effectiveness of GEM by promoting the expression of the deoxycytidine kinase and blocking the expression of the ribonucleotide reductase, a known resistance marker of GEM.[13,16] Furthermore, the ASS1 re-expression after management by arginine deprivation resulted in c-Myc stabilization that helped develop the resistance to arginine deprivation in pancreatic cancer cells. However, the addition of docetaxel enhanced c-Myc translocation into the nucleus and regulated the expression of the nucleotide/GEM transporter, and the human equilibrative nucleoside transporter 1, indicating that

combined treatment with ADI-PEG20 and docetaxel potentiates the anticancer activities of GEM by increasing GEM uptake *in vitro* and *in vivo*.[13]

Besides, ADI-PEG20 also contributed to the increase in radiation effects by triggering the endoplasmic reticulum stress pathway, leading to apoptosis in pancreatic tumor cells. The combination of ADI-PEG20 with radiation enhanced the expression of endoplasmic reticulum stress proteins and increased the reactive oxygen species load, and significantly sensitized the ASS1-deficient pancreatic cell lines, PANC-1, MIA paca-2, AsPC1, and Capan1. However, this radiosensitization was not observed for ASS1-expressing cell lines, such as BxPC3 and SW1990.[14] Moreover, it was reported that a combination of arginine deprivation and histone deacetylase (HDAC) inhibition has better efficacy in suppressing ASS1-low PDAC tumor growth. The combination of panobinostat and ADI-PEG20 triggered the degradation of a key DNA repair enzyme C-terminal-binding protein interacting protein, resulting in DNA damage and apoptosis.[27]

NOS plays an important role in the production of NO by catalyzing arginine. It was reported that eNOS was overexpressed, during murine pancreatic tumorigenesis. The knockdown or inhibition of eNOS decreased the tumorigenic growth of PDAC cell lines and reduced the expression of Hras-GTP, CD31, and Ki-67.[18] In addition, eNOS has also been shown to be closely associated with pancreatic tumor vascularization and angiogenesis.[57] Hence, the therapeutic strategy combining arginine deprivation with the inhibition of NO production may be a valuable direction in the development of clinical application for patients with metastatic PDAC.

Currently, an increasing number of phase 1–3 clinic trials are being conducted to evaluate the efficacy of ADI-PEG20 therapy in patients with cancers, including HCC, melanoma, mesothelioma, and PDAC.[59-65] In a phase 1/1B single-arm clinical trial of advanced pancreatic cancer, the combination of ADI-PEG20, GEM, and nab-paclitaxel was well tolerated. Among the patients who were treated with GEM (1000 mg/m$^2$) and nab-paclitaxel (125 mg/m$^2$) for 3 of 4 weeks and intramuscular ADI-PEG 20 at 36 mg/m$^2$ weekly, the overall response rate and median progression-free survival time were 45.5% (5 of 11) and 6.1 months (95% CI, 5.3–11.2 months).[65] The most frequent adverse events were neutropenia, thrombocytopenia, leukopenia, anemia, peripheral neuropathy, and fatigue. In addition, a series of clinical trials of ARG therapy were also carried out and showed desirable clinical results in cancer patients.[66-69] For example, the progression-free survival was significantly prolonged in advanced HCC patients who were treated with an adequate arginine depletion at a weekly dose of 1600 U/kg of pegylated recombinant human arginase (PEG-rhArg) for >2 months compared to those treated for 2 months or less (6.4 *vs.* 1.7 months; $P = 0.01$). Besides, the overall quality of life of the enrolled patients was well preserved and the PEG-rhArg1 treatment was well tolerated with a good toxicity profile.[68] Furthermore, targeting arginine metabolism, with therapeutic ADI or ARG, can be more effective in arginine auxotrophic tumors that are ASS1-negative.[69-71] Thus, further investigations are required to determine if ASS1 expression could be used as a predictive biomarker for arginine deprivation therapy.

## Arginine Deprivation Therapy Resistance

Drug resistance is an intractable obstacle in cancer therapy. The mechanism of drug resistance to arginine deprivation has been studied recently, and mainly focused on ASS1 re-expression, metabolism reprogramming, and antibody production.

In pancreatic cancer, the long-term treatment of pancreatic cancer cells by arginine deprivation resulted in a higher expression level of ASS1 compared with the non-treated pancreatic cancer cells; however, the long-term ADI treated cells exhibited a higher cell death rate.[13] The mechanism of ASS1 re-expression in pancreatic cancer remains unclear. In melanoma, ADI-PEG20 could activate the Ras/PI3K/ERK pathway, and stabilize c-Myc, which consequently induced the transcriptional expression of ASS1.[72] Moreover, ASS1 has a lower promoter methylation level and a high protein expression level in ADI-PEG20 resistant mesothelioma cells (APRCs) compared with ADI-PEG20 sensitive mesothelioma cells (APSCs).[16]

Metabolic reprogramming was also shown to be involved in the resistance to arginine deprivation. Through metabolomic and transcriptomic analyses, researchers found that compared with the APRCs, APSCs had lower acetylated polyamines and increased enzymes that are involved in polyamine catabolism, especially ornithine decarboxylase 1 (ODC1).[16] Further research suggested that ASS1-deficient cells are synthetically lethal with polyamine inhibition, a characteristic that was not observed in ASS1-proficient cells. Numerous studies have shown that polyamines can regulate the expression of several oncogenes.[73] ODC1 inhibition with DFMO modulated the pancreatic components of the ODC pathway, decreased proliferation, and increased the expression of p21 and p27.[74] However, it was found that targeting with DFMO had mixed clinical success due to pancreatic tumor escape mediated by an undefined transport system. The combination of DFMO with a PTI significantly reduced PDAC cell viability and prolonged survival of tumor-bearing mice.[40,75] Thus, the combination of arginine deprivation and an inhibition of polyamine biosynthesis and transport might be exploited clinically.

In addition to the polyamine metabolism, ADI-PEG20 resistant cells were found to exhibit enhanced glycolysis with high levels of lactate dehydrogenase A and glucose transporter 1, and a low level of pyruvate dehydrogenase in melanoma.[76] Notably, the arginine deprivation that is induced by ADI-PEG20 resulted in the reprogramming of glutamine and glucose metabolisms in leiomyosarcoma cells. Arginine deprivation inhibits aerobic glycolysis by decreasing the level of glycolytic enzymes and metabolites and enhancing serine and glycine biosynthesis through upregulation of the expression of phosphoglycerate dehydrogenase (PHGDH). Besides, arginine deprivation promotes glutamine uptake, utilization, and oxidative phosphorylation via the upregulation of the level of

expression of glutaminase (GLS) and glutamate dehydrogenase. Importantly, the combination of ADI-PEG20 with an inhibition of serine biosynthesis that was induced by PHGDH inhibitor or by the glutamine metabolism inhibition with a GLS inhibitor caused a significant synthetic lethality.[77] Though it is rarely reported that arginine deprivation causes glucose and glutamine metabolic reprogramming in pancreatic cancer, we believe that targeting these metabolic characteristics can be a potential combination treatment, based on arginine deprivation.

Although ADI conjugation with PEG reduced its immunogenicity, anti-ADI antibodies were still observed in patients, which might be related to ADI exposure time and concentration.[61] In addition, the high level of endogenous ARG2 in lung cancer cells could impede the antitumor effect of pegylated ARG1, causing resistance to ARG1 treatment. Furthermore, the silencing of ARG2 re-sensitized resistant tumor xenografts to ARG1 treatment.[78] Therefore, it is necessary to perform studies that clarify the impact of arginine deprivation on pancreatic cancer cells and investigate the mechanism of resistance to arginine deprivation, which might be influenced by the genetic background, epigenetic status, or metabolic factors.

## Arginine Metabolism in Immunotherapy

The immune checkpoint proteins in tumor-infiltrating T cells, including programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), are usually upregulated and, respectively, bind to their ligands PD-L1 (B7-H1)/PD-L2 (B7-DC) and CD80/86, which downregulates T cell response. Tumor cells can escape immune surveillance by upregulating immune checkpoint ligands, such as PD-L1. Therefore, it has been a research focus to block immune checkpoint proteins to activate antitumor immune activity in cancer immunotherapy and drug development.[79]

In recent years, the association between tumor metabolism and immunotherapy has attracted extensive attention. T cells could exhibit an impaired proliferation, a reduced interferon-γ release, and a PD-1 upregulation in response to antigen stimulation under low arginine conditions. It was reported that acute myeloid leukemia (AML) blasts inhibited T cell proliferation due to the high ARG2 enzyme activity, while the inhibition of arginine catabolism by targeting ARG2 enhanced T cells immunotherapeutic responses. Furthermore, the inhibition of the arginine metabolism could enhance CAR-T cell cytotoxicity against AML cells, leading to a significant reduction in viable AML cells.[80]

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population consisting of early myeloid progenitor cells, naive granulocytes, macrophages, and dendritic cells at different stages of differentiation. MDSCs can induce the immunosuppression of T cells via multiple mechanisms, involving the production of ARG1/2 and the upregulation of iNOS, which block the translation of T cells' CDζ, suppress T cell proliferation, and promote T cell apoptosis. In murine pancreatic tumors, there is a pronounced accumulation of MDSCs that have granulocytic ($CD11b^+Gr1^+Ly6C^{-/low}$) and monocytic origin ($CD11b^+Gr1^+Ly6C^{high}$), and that were reported to exert their immunosuppressive functions via the production of NO and the upregulation of ARG1. Approximately 88% of MDSCs from Panc02 xenograft tumors expressed ARG1 and 66% were positive for an inducible iNOS that catalyzes the production of NO. In *ex vivo* co-cultures, MDSCs that were isolated from tumors inhibited the proliferation of T cells that were isolated from spleens of PDAC-bearing mice. Furthermore, the expression of the tumor necrosis factor-alpha, interleukin-6, interleukin-13, VEGF, and transforming growth factor-beta was found to be increased in co-culture supernatants. MDSC was also found to be associated with an increased intratumoral VEGF concentration during PDAC progression.[81] Moreover, patients with oligo-metastases of various cancer types have elevated granulocytic MDSCs and certain subsets of monocytic MDSCs.[82,83]

In pancreatic cancer patients' tissue, neutrophil-like MDSCs (nMDSCs), but not monocyte-like MDSCs (mMDSCs), were found to be significantly increased. The author discovered that $CD13^{high}$ nMDSCs express higher levels of CD11b, CD33, CD16, and ARG1, but a lower level of CD66b compared with $CD13^{low}$ nMDSCs. Notably, $CD13^{high}$ nMDSCs suppressed alloreactive T cell response via an ARG1-related mechanism, resulting in immune escape and promotion of PDAC perineural invasion.[84] Thus, the elimination of MDSCs could be a promising strategy to enhance the effectiveness of cancer therapy. However, an antibody-based selective depletion of MDSCs remains elusive due to the high heterogeneity of MDSCs. As the immune suppressive factor, ARG has been a potential target in cancer immunotherapy. Several clinical trials have been conducted to explore the antitumor effect of a combination of ARG inhibitor with immune checkpoint inhibitors, such as pembrolizumab, that targets the PD-1 receptor.[80]

It was reported that the patients with ipilimumab and pembrolizumab resistant melanomas show complete remission at all stages of the disease, after a 30-month ARG (BCT-100) therapy that induced a persistent depletion in systemic arginine.[69] Thus, appropriate conditions are required to be determined for the arginine deprivation and ARG inhibition therapies. Arginine deprivation by ADI-PEG20 or PEG-rhArg in solid tumors including melanoma, HCC, and PDAC reveals an exact therapeutic effect, while ARG inhibition should be considered for applications in tumors within accumulated MDSCs, especially $CD13^{high}$ nMDSCs. However, it is also necessary to evaluate the antitumor effect of ARG inhibitor in immunotherapy-resistant solid tumors, especially pancreatic cancer and other metastatic cancers.

In general, arginine metabolism facilitates pancreatic cancer growth and progression. Arginine deprivation therapy for ASS1 low-expression PDACs and a combination therapy based on arginine deprivation appear to be potential therapeutic strategies in the future treatment of pancreatic cancer patients. In addition, the resistance mechanism of arginine deprivation remains to be further studied in pancreatic cancer. Moreover, the MDSCs with

enhanced arginine metabolism play a crucial role in the immunosuppression of T cells. These suggested that we should fully assess the tumor status, including, but not limited to the expression of ASS1 and accumulation of MDSCs while considering conducting a clinical trial, and develop appropriate metabolic therapies.


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## Conflicts of interests

None.

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