Cancer Letters 364 (2015) 1–7

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

# Targeting arginine metabolism pathway to treat arginine-dependent cancers

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ARTICLE INFO

*Article history:*
Received 22 January 2015
Received in revised form 28 March 2015
Accepted 19 April 2015

*Keywords:*
Argininosuccinate synthase 1
Arginine deprivation
Arginine deiminase
Arginase I
PEGylation

ABSTRACT

The significant disparities in metabolism between tumor and normal cells have inspired the development of metabolism-based anti-tumor therapeutics. Arginine is a semi-essential amino acid because normal cells can not only synthesize arginine *de novo* but also take up extracellular arginine. Several types of tumors have abnormalities in arginine metabolism enzymes and completely rely on extracellular arginine to support necessary biological processes. This property is referred to as arginine auxotrophy. Taking advantage of characteristic arginine auxotrophy in tumors, arginine deprivation, which is generally induced by the use of arginine deiminase (ADI) and arginase I, has been investigated as a novel strategy for cancer therapy. Arginine deprivation demonstrated promising efficacy against arginine-auxotrophic tumors. By integrating perspectives from both clinical oncologists and laboratory scientists, this article reviews the important aspects of arginine deprivation as a promising anticancer therapy.



## Introduction

A newly discovered biological hallmark of cancer is energy reprogramming, which allows tumor cells to adjust their metabolic pathways to obtain the maximum energy supply for uncontrolled cell proliferation. Therefore, tumor cells acquire distinct metabolic characteristics from normal cells [1]. For instance, tumor cells may terminate unnecessary energy-consuming processes, such as synthesis of non-essential amino acids, and obtain these nutrients through extracellular reservoirs instead. The significant disparities in metabolism between tumor and normal cells have inspired the development of metabolism-based therapies for cancers [2].

Arginine is a type of multi-functional amino acid involved in the synthesis of many metabolites, such as nitric oxide, urea, ornithine and citrulline, and is involved in protein modification and immunoregulation [3–6]. It is also a semi-essential amino acid because normal cells can not only synthesize arginine *de novo* through the ornithine cycle but also uptake extracellular arginine. Argininosuccinate synthase (ASS), argininosuccinate lyase (ASL), and arginase and ornithine transcarbamylase (OTC) are the primary enzymes involved in arginine metabolism, among which ASS is the key enzyme [7]. Several types of tumors have abnormalities in these enzymes, particularly ASS, and completely rely on extracellular arginine to support necessary biological processes [8]. This effect is referred to as arginine auxotrophy [8]. Taking advantage of characteristic arginine auxotrophy in tumors, arginine deprivation has been investigated as a novel strategy for cancer therapy and showed promising efficacy against arginine-auxotrophic tumors [9–11]. In fact, as early as the 1930s, arginine-rich feed was observed to promote the growth of xenograft tumors, while feed without arginine inhibited tumor growth. These data implied the presence of arginine-dependent tumor growth [12–14]. Later, a number of studies demonstrated that arginine deprivation using enzymes that degrade extracellular arginine eventually led to tumor cell death [15–19]. In recent years, the potential clinical applications of arginine deprivation for the treatment of hepatocarcinoma (HCC), malignant melanoma (MM), prostate cancer and acute lymphoblastic leukemia (ALL) have been investigated and proven to be feasible and effective [20–25]. This review summarizes the major findings and progress in the utilization of arginine deprivation as a promising strategy for the treatment of cancers.

## Argininosuccinate synthase 1 (ASS1) deficiency in tumors

Compared to ASL and OTC, more attention has been focused on ASS1. The human *AAS1* gene, which is located at 9q.34.11–9q34.12, has 16 exons and a 1236 base-pair segment of open reading frame (ORF). Upstream of the *ASS1* gene, there is an 800-bp long promoter region containing a TATA box, E-box and GC box, suggesting that *ASS1* expression can be modified by transcription factors such as

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http://dx.doi.org/10.1016/j.canlet.2015.04.020
0304-3835/

c-Myc, HIF-α and SP4 [26,27]. In some tumors, such as malignant pleural mesothelioma, lymphoma, myxofibrosarcomas and glioblastoma, *ASS1* could be silenced by hypermethylation of its promoter region, and demethylation reactivated *ASS1* expression [28–31]. In addition to the classical promoter region, a cAMP response element has been identified and is located approximately 10 kb upstream of *ASS1*, which indicates that *ASS1* may also be regulated by TSE-1 [32]. The human ASS1 protein consists of a nucleotide-binding domain, a synthase ribbon and a c-terminal helix region, and it has 412 amino acids in total. The ASS1 protein is a tetramer, and its crystal structure has been identified [33].

ASS1 is highly expressed in normal tissues but heterogeneously expressed in tumors. The expression of ASS1 was first investigated in 2004 by Dillion et al. in different tumor samples using a monoclonal antibody [8]. These researchers found that ASS1 was completely deficient in HCC, MM and prostate cancer and partially deficient in breast cancer, renal cancer and sarcoma, while it was highly expressed in colorectal cancer, gastric cancer and lung squamous cell cancer [8]. Subsequently, more studies have been conducted to determine the expression of ASS1 in tumors using commercial anti-ASS1 antibodies. In these studies, ASS1 deficiency was determined at the mRNA, DNA and protein levels by RT-PCR, methylation-specific PCR and immunohistochemical staining, respectively (Table 1). A number of other tumors such as non-Hodgkin's lymphoma, Hodgkin's lymphoma, pancreatic carcinoma, MM, osteosarcoma and malignant pleural mesothelioma were also found to be deficient in ASS1 expression (Table 1). Recently, we determined the expression of ASS1 in tumor tissues obtained from 149 breast cancer patients using the ASS1 antibody and found that 63.8% (95/149) of breast tumors exhibited low or no cytoplasmic staining of ASS1 [35].

**Table 1**
Summary of ASS1 expression and its prognosis value in tumors.

| Tumor | ASS1 deficiency[a] | Prognosis value |
|---|---|---|
| Bladder cancer [34] | 45% (191/478) | Positive correlation |
| Breast carcinoma [8,35] | 9% (5/56) | NA |
| | 63.8% (95/149) | Positive correlation |
| Colorectal carcinoma [8] | 2% (1/46) | NA |
| Esophageal Carcinoma [36] | 19% (6/32) | Positive correlation |
| Glioblastoma [30] | 36% (8/22) | NA |
| Head and neck carcinoma [37] | 56% (41/73) | Negative correlation |
| Hepatocellular carcinoma [8] | 100% (51/51) | NA |
| Hodgkin's lymphoma [29] | 97% (173/179) | NA |
| Kidney carcinoma [8,38] | 29% (6/21) | NA |
| | 100% (98/98) | NA |
| Lung squamous carcinoma [8] | 12% (3/26) | NA |
| Malignant pleural mesothelioma [28,39] | 63% (52/82) | NA |
| | 46% (98/214) | NA |
| Malignant melanoma [8,40] | 100% (119/119) | NA |
| | 62.9% (17/27) | NA |
| Myxofibrosarcoma [31] | 44% (40/90) | Positive correlation |
| Non-Hodgkin's lymphoma [29] | 95% (246/258) | NA |
| Nasopharyngeal carcinoma [41] | 52% (64/124) | Positive correlation |
| Osteosarcoma [42] | 63%(39/62) | Positive correlation |
| Ovarian cancer [8,43] | 4% (2/45) | Positive correlation |
| | 43% (23/54)[b] | Positive correlation |
| Pancreatic carcinoma [44] | 87% (41/47) | NA |
| Prostate carcinoma [24] | 100% (88/88) | NA |
| Retinoblastoma [45] | 0% (0/20) | NA |
| Sarcoma [8] | 22% (6/27) | NA |
| Seminoma [8] | 17% (2/12) | NA |
| Small cell lung carcinoma [46] | 44% (7/16) | NA |
| Stomach carcinoma [8] | 0% (0/6) | NNA |

[a] ASS1 deficiency indicates the ratio of patients with absent/low expression of ASS1 at protein, mRNA and DNA levels, as determined by immunohistochemical staining, RT-PCR or methylation-specific PCR respectively, to all the patients enrolled.
[b] *ASS1* deficiency was evaluated by methylation-specific PCR, while all others summarized in this table were evaluated by immunohistochemical staining.
NA, not applicable.

Although ASS1 primarily functions as an enzyme, its non-enzymatic functions, such as tumor suppression, have been discovered. For instance, *ASS1* down-regulation was associated with lymphatic dissemination in patients with esophageal adenocarcinoma [36]. Reduced *ASS1* expression was also significantly correlated with the development of pulmonary metastasis after surgery and poor prognosis in patients with osteosarcoma, while over-expression of *ASS1* inhibited tumor growth *in vitro* [42]. Moreover, ASS1 deficiency was identified in 51.6% (64/124) of patients with nasopharyngeal carcinomas (NPC), and it was associated with advanced tumor stage, high local recurrence rate and poor disease-free survival [41]. Coincidently, ASS1 deficiency was also linked to advanced stage and worse survival in patients with myxofibrosarcoma [31]. In ovarian cancer, *ASS1* methylation was associated with significantly reduced overall survival and relapse-free survival and contributed to treatment failure [43]. Recently, it was reported that 40% of bladder tumors exhibited ASS1 deficiency, which was associated with worse disease-free and metastasis-free survival. In addition, epigenetic silencing of *ASS1* consistently promoted tumor proliferation and invasion [34]. Our recent data demonstrated that the lack of ASS1 expression was associated with poor prognosis in breast cancer, and knockdown of *ASS1* expression with *shASS1* promoted breast cancer proliferation and invasion [35]. Consistent with these findings, re-introduction of *ASS1* impeded tumor angiogenesis, tumor proliferation and migration *in vitro*, further indicating that *ASS1* might be a novel tumor suppressor [31]. It was reported that enhanced synthesis of nucleosides induced by the addition of extracellular arginine might contribute to the aggressive phenotype of ASS1-negative tumors. However, the underlying mechanisms need further investigation [47]. Interestingly, contradictory to the above data, high ASS1 expression was associated with unfavorable disease-free survival in head and neck carcinoma [37].

## Strategies for arginine deprivation

As indicated in Fig. 1, arginine deprivation could be achieved by utilizing enzymes that catalyze plasma arginine into other metabolites. Theoretically, a series of enzymes such as arginine deiminase (ADI), arginase, arginine decarboxylase (ADC) and nitric oxide synthases (NOS) are potentially useful for arginine deprivation [48]. However, due to the requirements of *Km* values, substrate specification, optimal pH and stability of enzymes for *in vivo* use, ADI and arginase are most commonly used for arginine deprivation.

ADI was first discovered to be responsible for transforming arginine into citrulline in *Bacillus pyocyaneus* [49]. Later, it was also found in bacteria, yeast and mycoplasma. As early as the 1960s, the anti-tumor activity of ADI obtained from *Mycoplasma* (*PPLO* strain) was first demonstrated in murine lymphoma *in vitro* but not *in vivo* [50,51]. Afterwards, the anti-tumor effect of ADI purified from *Mycoplasma* was further demonstrated in several different types of tumors *in vitro* and *in vivo*, especially in human HCC [52]. Interestingly, medium contaminated with *Mycoplasma arginini* also inhibited the proliferation of Rous sarcoma virus-transformed rat liver cells [53,54]. Moreover, *Mycoplasma* consists of a large variety of strains that produce different types of ADI, which results in diverse anti-tumor activity and therapeutic applications [55]. Currently, the commonly used ADI protein is derived from *M. arginini* and consists of two subunits with a total molecular weight of approximately 43 kDa. At physiological status, 70% of the enzyme activity of ADI could be reserved with an optimal pH of approximately 6.0–7.5 at 50 °C. Although the *Km* value of ADI for arginine (0.3 mM) is 30 times