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Modulation of arginine metabolic pathways as the potential anti-tumor mechanism of recombinant arginine deiminase

Li-Jiuan Shen, Karin Beloussow, Wei-Chiang Shen*

Department of Pharmaceutical Sciences, School of Pharmacy, University of Southern California, 1985 Zonal Avenue, 404B, Los Angeles, CA 90089-9121, USA

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Abstract

Arginine deiminase (ADI), currently in clinical trials, has various biological activities including anti-proliferation, anti-angiogenesis and inhibition of inducible nitric oxide synthase (iNOS). To recognize limitations and therapeutic applications, the mechanism of ADI modulation of arginine metabolic pathways was investigated. MCF-7 and A549 cells have notable different sensitivity to recombinant ADI (rADI) and express diverse argininosuccinate synthase (AS) activity, which regenerates arginine. Due to compartmentalization of arginine, utilization of arginine for protein synthesis occurs from either the intracellular arginine pool or the citrulline–arginine-regeneration pathway, whereas for polyamine synthesis, utilization is only from the intracellular arginine pool. Modulating AS activity or introducing rADI intracellularly to reduce intracellular arginine regeneration may expand therapeutic applications of rADI.

Keywords: Arginine deiminase; L-arg; De novo protein synthesis; Polyamine synthesis; Resistance

1. Introduction

To starve cancer cells through amino acid deprivation can be a strategy in cancer therapy. Specific amino acids, although not classified as essential, are required for the growth of certain tumor cells while normal cells can synthesize sufficient amounts for their own needs. For example, asparaginase has been used to deplete asparagine in the treatment of acute lymphoblastic leukemia and a few sub-types of non-Hodgkin's lymphoma because these cancers lack asparagine synthetase and are auxotrophic for asparagine [1]. Some cancers have an elevated requirement for arginine, such as metastatic melanoma and hepatocellular carcinoma. Arginine deiminase (ADI), the enzyme catalyzing the hydrolysis of arginine to citrulline, is currently being used as a chemotherapeutic agent against these arginine-requiring cancers and has gained much attention in recent clinical trials [2]. The sensitivity of various cell lines to ADI has been reported to be dependent upon the expression of argininosuccinate synthase (AS), the rate-limiting enzyme in the conversion of citrulline into arginine [3].

* Corresponding author. Tel.: +1 323 442 1902; fax: +1 323 442 1390.
E-mail address: weishen@usc.edu (W.-C. Shen).

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AS expression had not been detected in specimens tested from melanoma, hepatocellular and prostate carcinomas, and it was then concluded that this resulted in the arginine auxotrophy [4]. However, AS expression has been detected in some tested human tumor biopsy specimens which might cause the tumors to be refractory to ADI therapy. Therefore, ADI might be a promising enzyme in the treatment of tumors without AS expression, however, ADI has shown its anti-proliferative and anti-angiogenic activities in a variety of cancer cells and endothelial cells in vivo and in vitro [3,5,6] and the exact mechanism of its anti-tumor activity remains unclear. Therefore, an investigation of ADI-regulated cellular functions is important in order to recognize the applications and limitations of this therapeutic enzyme, and in this study we are the first to present the possible varied mechanisms of rADI in cancer therapy.

Since arginine is involved in several pathways for regulation and maintenance of cellular functions, such as protein synthesis, polyamine synthesis, and nitric oxide (NO) production [7], ADI may modulate these physiological pathways. We have previously reported that ADI is a selective modulator for NO production via inducible (iNOS) and endothelial (eNOS) nitric oxide synthases [8]. In this paper, we further investigate ADI and its effect on the regulation of cellular protein and polyamine synthesis. The anti-proliferative and anti-angiogenic effects of ADI might be a consequence of protein synthesis that involves cell growth and tumorigenesis [9] and polyamine synthesis that involves cell proliferation and differentiation [10]. Our results also provide important information regarding the various arginine compartments, such as extracellular, intracellular, and regenerated arginine pools, and their roles in protein and polyamine synthesis.

2. Materials and methods

2.1. Materials

rADI was prepared and purified to homogeneity in our laboratory as previously described [5]. For the de novo synthesized protein assay, [^{35}S]methionine (1000 Ci/mmol) was obtained from Moravek Biochemicals (Brea, CA). Micro BCA protein assay reagent kit was obtained from Pierce (Rockford, IL). All other chemicals and reagents were purchased from Sigma Chemical Company (St Louis, MO).

2.2. Cell culture

Human mammary adenocarcinoma (MCF7) and human lung carcinoma (A549) cells were obtained from American Type Culture Cell (ATCC) (Manassas, VA) and maintained in medium recommended by ATCC, supplemented with 10% fetal bovine serum. All cell culture reagents and L-arg-free/L-cit-free MEMα + medium were products of GIBCO-BRL (Carlsbad, CA), except methionine-free DMEM, which was a product of MP Biomedicals (Aurora, Ohio). For experiments in this report, the L-arg-free/L-cit-free MEMα + medium is designated as 'L-arg-free medium'. L-arg-free medium supplemented with 1 mM L-arg is designated as 'control medium' and when supplemented with 1 mM L-cit/1 mM ammonium chloride, designation is 'L-arg-free/L-cit-supplemented medium'.

2.3. [^{35}S]methionine incorporation into de novo synthesized protein

MCF-7 and A549 cells were seeded in 6-well plates in control medium. The following day, cells were cultured in control (with and without 1 mU/ml rADI), L-arg-free/L-cit-supplemented and L-arg-free medium. On day 3, to reduce background incorporation of methionine into newly synthesized proteins, the cells were pre-incubated for 1 h in the respective rADI-pre-treated methionine-free medium. Post the 1 h pre-incubation, cells were incubated with 2.5 μCi [^{35}S]methionine for one additional hour. The medium was then removed and the cells were washed with cold PBS and lysed with 1 ml 0.1% Triton-X. The cell lysates were mixed with 10% trichloroacetic acid (TCA) on ice for 30 min, and then centrifuged at 18,000*g* for 15 min. The resulting pellet was washed twice by centrifugation and resuspended in 5% TCA. The pellet was then dissolved overnight in 1 ml 0.1 N NaOH. Eight hundred microliters of NaOH digest was transferred to a scintillation vial and radioactivity counted by a scintillation counter. Protein concentration of NaOH digests was measured by Pierce