Chapter

Arginine Metabolism: An Enlightening Therapeutic Attribute for Cancer Treatment

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Abstract

Arginine is well known semi-essential amino acid used in protein biosynthesis through several metabolic pathways. It is majorly obtained from nutrients sources and synthesized by the urea cycle in the body using citrulline. Arginine found to be involved in several mechanisms including; hormone synthesis, cell division, activation of the immune system, ammonia disposal and wound healing and also in the production of nitric oxide (NO) and polyamines. During cancer persistence, the biosynthesis of arginine is not sufficient to compensate for their higher nutritional requirements but extracellular availability of arginine is also required. Therefore, the consequences of arginine deprivation may represent a novel targeting therapy to cure cancer. The impact of different arginine deprivation agents and their mechanism of action always found to be correlated with NO and polyamine levels. Arginine deprivation strategy to hamper the proliferation of cancerous cells and their migration is represented as a new approach to cure cancer by inhibiting the argininosuccinate synthetase1 (ASS1) expression and NO and polyamines production. ASS1 is the first key enzyme that converts citrulline to arginine and numerous tumors such as hepatocellular carcinoma, melanoma, mesotheliomas and renal cancer do not express ASS1 and main focused enzyme for cancer treatment. Degradation of arginine by the enzyme arginine deiminase (ADI) specifically triggers the arginine elimination and inhibition of cancer migration. Though, ADI is a microbial enzyme but has a high affinity to arginine and converts arginine into citrulline and NH_3. This produced citrulline can be recycled back to arginine in normal cells where ASS1 expression is very high in comparison to ASS1-negative tumor cells. A modified form of ADI with pegylate (ADI-PEG20) has been formulated which showed both in-vivo and in-vitro activity against hepatocellular carcinoma and melanoma by inducing apoptosis. In this chapter, we have majorly discussed arginine production with different pathways and how its degradation into other metabolic active compounds involved in cancer treatment. Moreover, how arginine deprivation is directly taking part in the inhibition of cancer cell proliferation and its migration.

Keywords: arginine, metabolic pathways, arginine deprivation, cancer therapy

1. Introduction

Arginine is essential for microbes and semi-essential for eukaryotes which play numerous crucial roles in cellular metabolism. The impact of arginine always considered as a nonessential amino acid because a cell can synthesize arginine its

own as per the requirement. But, during its limitation in the cells, it is necessary to acquire arginine from outside environment and this condition denoted arginine as a conditionally essential amino acid. Majorly, arginine is produced by two ways; from food sources and biosynthesized through urea cycle in the kidney [1]. The biosynthesis of arginine represented the conversion of citrulline to arginine by the enzymes arginosuccinate synthetase1 (ASS1) and arginosuccinate lyase (ASL). The role of enzyme ASS1 is the conversion of citrulline and aspartic acid to arginosuccinate, which then directly converted to arginine and fumaric acid by the enzyme ASL [2]. In case of bacteria, ornithine also indicated as a substrate to synthesize arginine by the enzyme ornithine transcarbamylase (OCT) [3]. Arginine is a precursor molecule for the formation of amino acids such as proline, glutamate and arginine itself and several other components like succinate, nitrate, nitrite, nitric oxide (NO), ammonia and CO_2. It acts as an intermediate in urea cycle and precursor molecules for polyamine, creatine and proteins biosynthesis [4]. Arginine becomes necessary for growth and promotes wound healing by stimulating the release of growth hormones such as insulin-like growth factor-1, insulin and prolactin and also has several immunomodulatory effects such as stimulation of T cells, natural killer cell and enhances pro-inflammatory cytokine levels [5]. Thus, arginine deprived cancer cells can be rescued by activating immunity and increasing the flux of arginine through urea cycle [6]. When a cell is under stress or need to proliferate like tumor cell, then the requirement of cellular components such as citrulline, nitric oxide and polyamine levels get increase. Therefore arginine synthesis and degradation tremendously increase in cancer cells [7]. Arginine depletion is one of the most accepted way to cure tumor cells which are auxotrophic (dependent on uptake of extracellular arginine) to arginine. Some tumor cells adapted with downregulated arginine metabolizing enzymes for inhibiting the production of arginine from the substrates and become arginine auxotrophic [8]. Therefore, during cancer some nonessential amino acids turned in to the essential and cancer cell becomes auxotrophic for these [9]. As we all know that cancerous cells are associated with very high survival rates, therefore, some significant improvements are required for early detection and treatment of cancer. The idea for cancer treatment open the door for some most advanced approaches including; hormone therapy, stem cell therapy, immunotherapy and amino acid deprivation therapy [6, 10, 11]. One of the most capable amino acid deprivation therapy is arginine deprivation where arginine-depleting agents are the main focused and depletion of arginine harms the ability of cancer cell metastasize. The mechanisms of arginine impairment are still not clear hence, in this chapter we will try to give a brief discussion about the different biosynthesis and catabolic pathways of arginine. How arginine deprivation can be focused for cancer therapy for both arginine auxotrophic and non-auxotrophic cancerous cells with different mechanism of actions. Moreover, we will discuss the impact of arginine deprivation in cell migration through different intermediates production such as polyamines and NO.

2. Arginine biosynthesis pathways

Arginine is synthesized from citrulline by the key enzymes ASS1and ASL of the urea cycle which also called ornithine cycle and then released into the bloodstream (**Figure 1a**). In large animals, citrulline is produced majorly from NH_3, CO_2 and ornithine by the enzymes OTC and carbamylphosphate synthetase I (CPS1) in the small intestine. Citrulline is also recycled to arginine when both argininosuccinate ASS1and ASL are present in the same cell and take part in to the citrulline-nitric oxide cycle [12]. In contrast arginase and nitric oxide synthetase use arginine as a

common substrate and always compete for this substrate [13]. Arginine biosynthesis exhibits diverse pattern of gene organization in bacteria, mammals and plants and uses different set of enzymes which catalyze reactions for the formation of a key intermediate "ornithine". Additionally, glutamate is also utilized as the precursor for ornithine synthesis using some intermediates of the urea cycle [13, 14]. Extracellular arginine is also a source for ornithine synthesis in cells by enzyme arginase 1 [12]. In bacteria and plants, ornithine is synthesized from glutamate in five enzymatic steps initiated by the acetylation of glutamate by N-acetylglutamate synthase and called N-acetylglutamate synthase pathway (**Figure 1b**) [15]. Here, first ornithine is converted to citrulline by ornithine carbamoyltransferase.

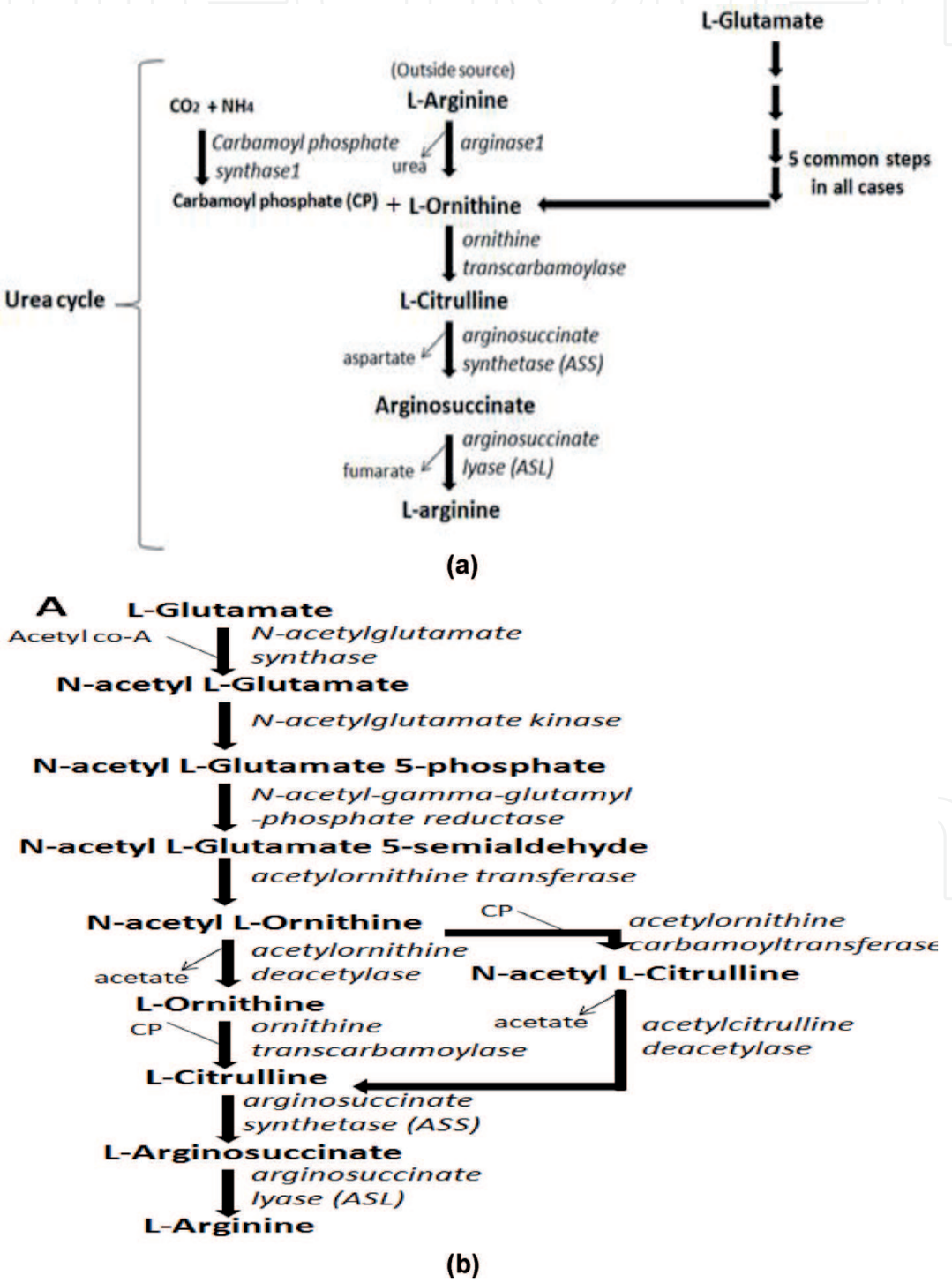

Figure 1.
(a) Arginine biosynthesis from glutamine and arginine itself in the urea cycle. (b) Key steps of the arginine biosynthesis form glutamine.

Enzyme ASS1 catalyzes the conversion of citrulline to aspartate and argininosuccinate which is further converted into arginine and fumarate by ASL [16, 17]. Ornithine can also be converted back to citrulline by arginine deiminase (ADI) pathway in bacteria [18] and by arginase1 pathway in mammals [19]. In both the cases citrulline is recycled back to arginine by ASS enzyme [15]. The ability to generate arginine from citrulline depends on the activity of ASS and ASL [20]. These two enzymes are tightly coupled for sensitivity of cells to arginine deprivation and their activity depends on their ability to regenerate arginine from the alternative sources [21].

3. Arginine catabolic pathways

There are enumerating pathways and enzymes to degrade arginine into other biomolecules and intermediates. Five main pathways including; arginine succinyltransferase (AST) (**Figure 2A**), arginine decarboxylase (ADC) (**Figure 2B**), Arginase1 (**Figure 2C**), citrulline- NO ((**Figure 2D**) and arginine deiminase (ADI) (**Figure 3**) were found to degrade arginine. These pathways are mainly focused by the researchers to study arginine degradation and find out its role in different cellular activities and ADI pathway has higher affinity for arginine among all of these pathways [22]. The essential site for arginine degradation in ureotelic organisms is the liver and second main site is the kidney where arginine is major converted into the polyamines, urea, creatine phosphate and NO and transported through bloodstream into the cells by cationic amino acid transporters (Melis et al, 2008). In bacteria arginine is degraded via three key pathways; (i) ADC pathway, here, arginine degradation is initiated by decarboxylation of arginine and form agmatine which further converted into putrescine by enzyme agmatine ureohydrolase. Putrescine is converted into γ- aminobutyric acid by putrescine transaminase and pyrroline dehydrogenase and ultimately converted into glutamate and succinate [23].

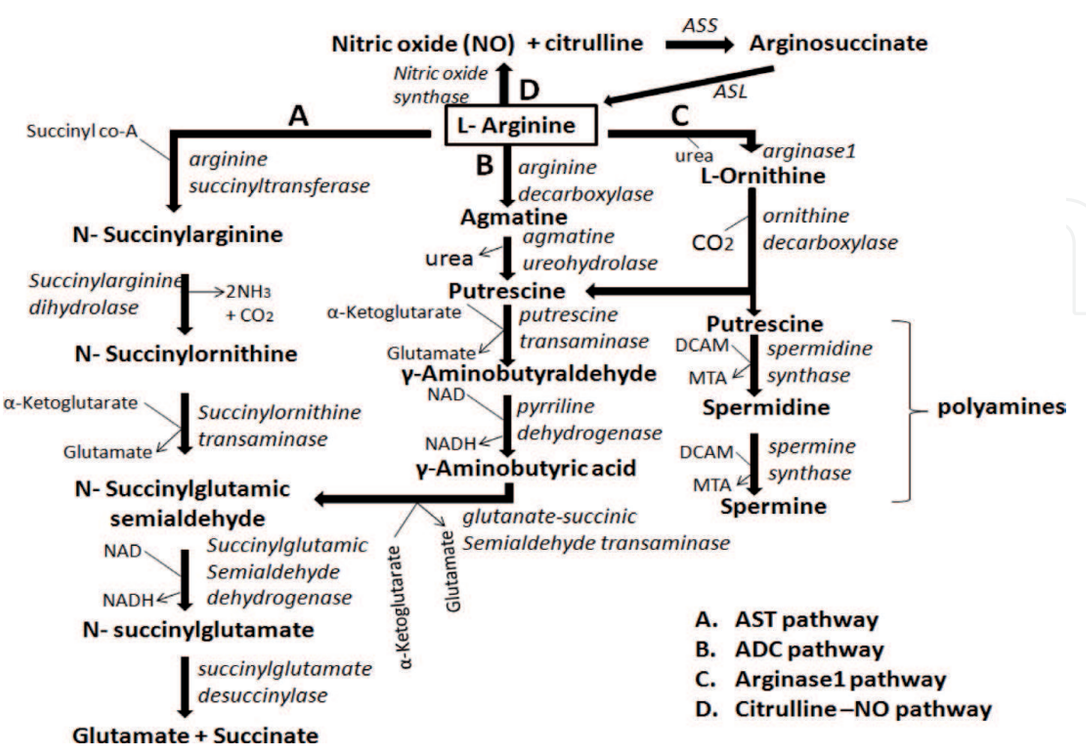

Figure 2.
Arginine biosynthesis by different metabolic pathways such as AST pathway (A), ADC pathway (B), arginase1 pathway (C) and citrulline-NO pathway (D).

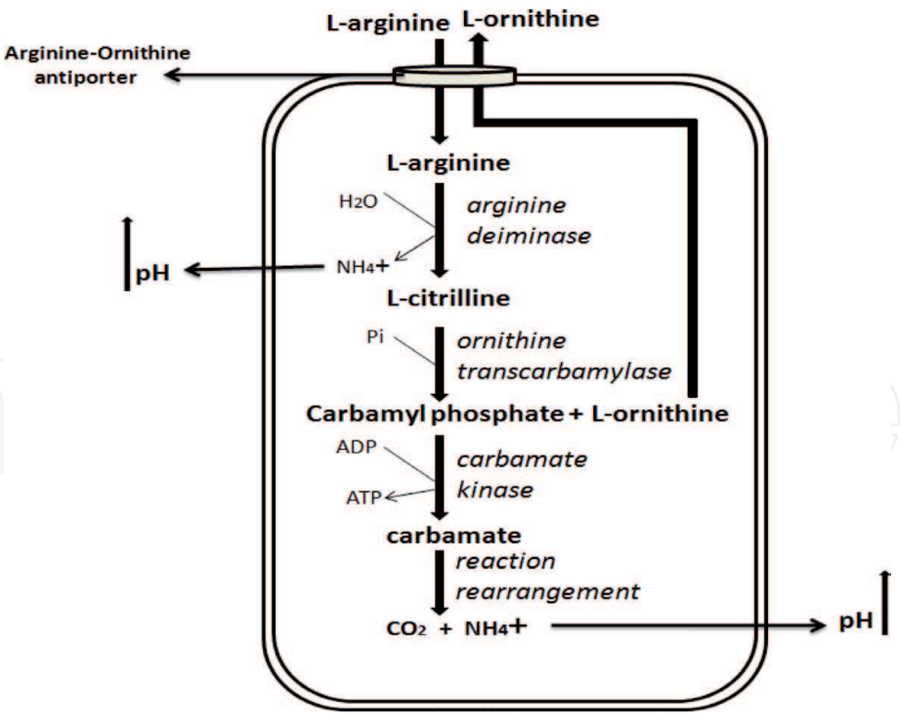

Figure 3.
degradation of arginine through ADI pathway in the bacterial system.

The enzyme arginine decarboxylase (ADC) is also considered as an important enzyme in bacteria [24], plants [25] and in mammalian systems [26]. The infusion of agmatine in the cerebral ventricles increases blood pressure and regulates the angiogenic activities [27]. (ii) AST pathway; here, arginine is degraded into glutamate, succinate and other intermediates. AST pathway is mainly activated for arginine degradation when nitrogen is limited for growth and contributes into the production of amino acids [28]. (iii) Arginase1 pathway; this pathway is activated when arginine concentration is excess in the media and urea and ornithine are produced by enzyme arginase1 during first enzymatic reaction [29]. Here, urea does not metabolize further and rapidly excreted into the medium. In arginase1 pathway, arginine used as the nitrogen and carbon sole sources and less than 3% of consumed arginine results in the formation of urea and 36% consumed by the route of putrescine and polyamine synthesis [30]. Polyamines produced by this pathway are polycations and interact with negatively charged molecules, such as DNA, RNA and also with proteins and involved in cellular growth, survival and proliferation [31]. Polyamines such as putrescine, spermidine and spermine are very tightly regulated by polyamine metabolic pathway [32]. These metabolites used by *H. pylori* to retard the expression of pro-inflammatory cytokines and prevent the immune response in stimulated macrophages [33] and also maintain the microenvironment around their cell in acidic condition for their survival using arginine [34]. Cancer and proliferative cells show high levels of polyamines and with this feature cancer cells maintain their proliferative properties [32] and high levels of polyamines were observed in cancerous cells [35]. It is proposed that both Gram negative and positive bacterial cells which contain unusually high AST and ADI level grow anaerobically in a complex acidic medium and both the enzymes help to raise the pH for the cell survival in the acidic environment [36]. Last but not least, arginine deiminase (ADI) pathway degrades arginine to ornithine, ammonia, and carbon dioxide and generates one mol of ATP by utilization of per mol of arginine [37]. A variety of bacterial cells; both gram positive and gram negative can catabolized arginine through ADI pathway [18]. Enzyme activity of ADI has been detected

in several lactic acid bacteria (LAB), *bacilli, clostridia, pseudomonads, aeromonads, mycoplasmas, halobacteria, and cyanobacteria* [36]. ADI pathway is completed by three key enzymes: arginine deiminase (ADI), ornithine transcarbamoylase (OTC), and carbamate kinase (CK) as shown in **Figure 3**. Moreover, in *Pseudomonas aeruginosa*, a fourth gene that encodes a transport protein to exchange arginine and ornithine for this pathway has been identified [37]. ADI pathway is most important for the bacterial cell survival in the acidic environmental condition because arginine degradation by ADI pathway produced ammonia that raises the cytoplasmic and extracellular pH and produced ATP use as the energy source for cell survival. In the absence of carbohydrate bacteria preferred arginine and utilize it by ADI pathway as an alternate energy source to engender energy for cellular growth [20, 38]. ADI pathway is regulated at transcriptional level and regulated by transcriptional regulator ArgR [3, 37]. Moreover, carbon catabolite repression (CCR) has also been confirmed for the expression of ADI pathway in various bacteria. CCR regulates the expression of arc operon with glucose and catabolite control protein A (CcpA) [39]. CcpA is a transcriptional regulators belonging to the Crp/Fnr family and regulates the expression by the binding with regulatory proteins to the cis-acting catabolite response elements (cre) located in the promoter regions [20].

4. Impact of arginine degradation in cancer therapy

Cancer cells need excess quantities of specific amino acids for their diverse metabolism rate for higher proliferation and become resist for some cell death signals. Identification of the metabolic dissimilarly between cancer cells and normal cells, cellular metabolism of cancer cells is a therapeutic target and focusing field of cancer research [40]. The deprivation of arginine inside cancer cells, which makes the cells auxotrophic, has been centered one of the novel approach for cancer treatment [22]. There are several targets have been reported which directly take part in cancer mitigation as discussed below;

5. Citrulline-NO cycle

Citrulline is well known as a byproduct of NO synthetase enzyme and can be recycled to arginine by the key enzymes ASS1 and ASL. Both these enzymes are strongly expressed in liver and kidney then the other cells and tissues. The citrulline-nitric oxide cycle stimulates the activation of cytokines such as interferon (IFN) [41] and enhances the expression level of ASS1 enzyme as noticed in mouse microglial cells [42] and human tumor cell lines [43]. Impaired NO production from citrulline has been reported as a vital factor for the abnormal proliferation of keratinocytes in psoriasis epidermis. Higher arginase I with induced NO synthetase inhibits the keratinocyte proliferation by eliminating the arginine availability [44]. Enzymes for arginine metabolism are the potent therapeutic targets to control NO and cancerous cell proliferation as shown in **Figure 4**. In citrulline-NO pathway, NO is synthesized from arginine by the three nitric oxide synthase (NOS) isoforms; endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) and maintain the citrulline -NO cycle in the functional cells [45]. NOS and arginase1 use arginine as same substrate but arginase1 down-regulate because NO production by competing with NOS for arginine [46]. Remarkably, iNOS and arginase1 activities are reciprocally regulated in the cancerous cells by the involvement of cytokines and this can be guaranteed for the optimum production of NO but not in immunostimulated macrophages [47].

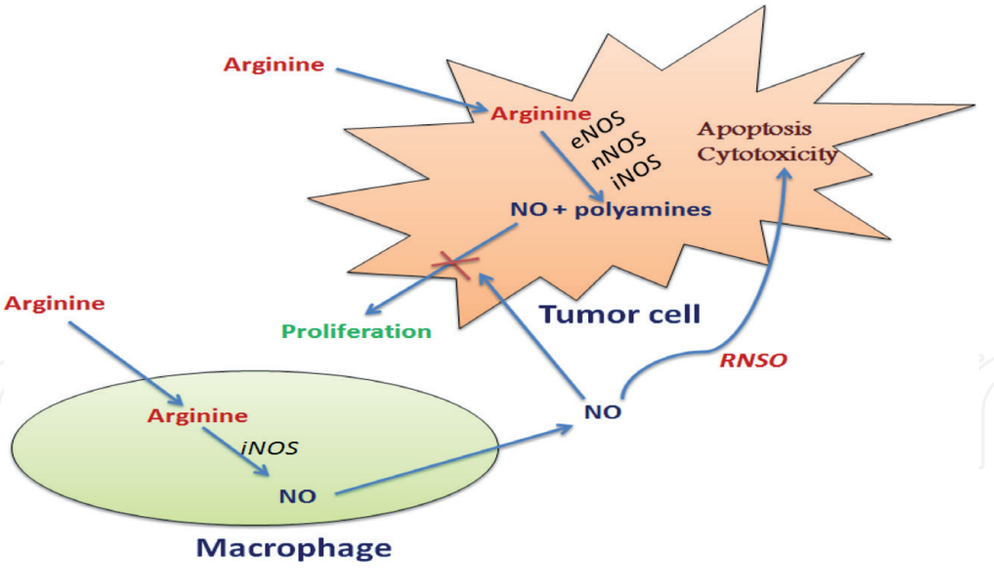

Figure 4.
Role of nitric oxide for tumor alimination.

6. Inhibition of ASS1 activity

The mechanisms which exhibited the loss of ASS1 activity are specifically depend on the type of cancerous cell and availability of arginine. ASS1 is a rate-limiting enzyme involved in arginine biosynthesis and has been investigated in numerous cancerous conditions such as melanoma [48], hepatocellular carcinoma [49] and pancreatic cancers [50], and the. ASS1-negative cancer cells are auxotrophic for arginine and exhibit sensitivity to arginine deprivation [51]. Cancerous cells have lack expression of ASS1 enzyme required for arginine biosynthesis which is an exogenous source for proteins synthesis and cellular growth [52]. Less ASS1 expression was recorded as a biomarker in cancer cell and for overall cellular functioning. The ASS1-deficient cancers with arginine auxotrophy have been initiated as the development of therapeutics by depriving arginine through degradation and trigger the apoptosis in arginine auxotrophic cancerous cells [53] as shown in **Figure 5**. The low levels of acetylated polyamine metabolites were found in arginosuccinate

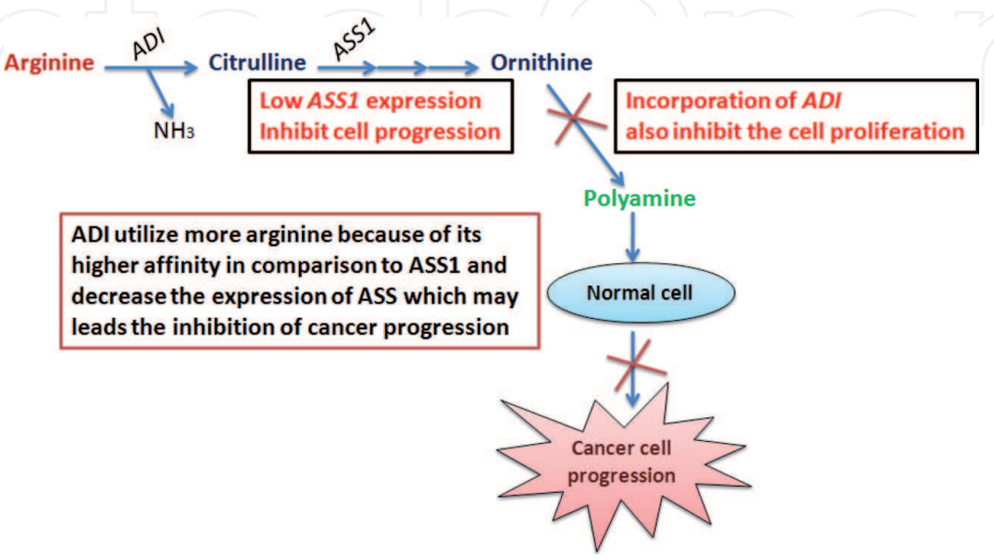

Figure 5.
Impact of gene ASS1 expression for cancer progression and proliferation and its down-regulation by ADI enzyme.

synthetase-deficient cells, pinpointing the reduction in catabolism and increase the expression of polyamine biosynthetic enzymes. This metabolic reprogramming elucidates a synthetic lethal interaction between arginosuccinate synthetase loss and polyamine metabolism, which could potentially be exploited for the treatment of arginosuccinate synthetase-negative cancers [54]. The reason for down-regulation of arginosuccinate synthetase in cancer cells is not cleared properly but always remains the center of interest among the cancer researchers [55].

7. ADI obstructed the angiogenetic activity

The enzyme ADI inhibits the tumor growth not only by depletion of arginine but also by suppression the angiogenic activity via less NO production [20] as shown **Figure 6**. ADI has an also strong capability to deplete arginine from plasma and inhibit NO production which resulting an effective inhibitory role of ADI in NO-mediated angiogenesis [56]. During in vitro study, the anti-angiogenic activity of ADI to inhibit micro vessel tube formation and migration in endothelial cell cultures was reported by Beloussow et al. [57]. Arginine depletion with the treatment of ADI enzyme also alters the level of proline, polyamines, glutamate and succinate. Polyamines are essential for tumor proliferation and their less production directly affects angiogenesis. Mycoplasma-derived ADI-PEG20 is majorly focused and most commonly used as a potential therapeutic agent for clinical investigation with different anti-neoplastic activity [58]. Mechanistically, ADI is capable of inhibiting the metabolic activity of cancerous cells and take parts in autophagy and apoptosis of auxotrophic cells. [59].

Induction of apoptosis

Arginine limitation has also been recorded to induce apoptosis which leads cell death in ASS1-negative tumor [60]. Even though, the signaling pathway for apoptosis is not clear yet, but it has been reported that apoptosis induced by arginine deprivation can be activated via caspase-dependent/independent pathways [1]. The limitation of arginine in ASS1-negative mesothelioma cells induced apoptosis via

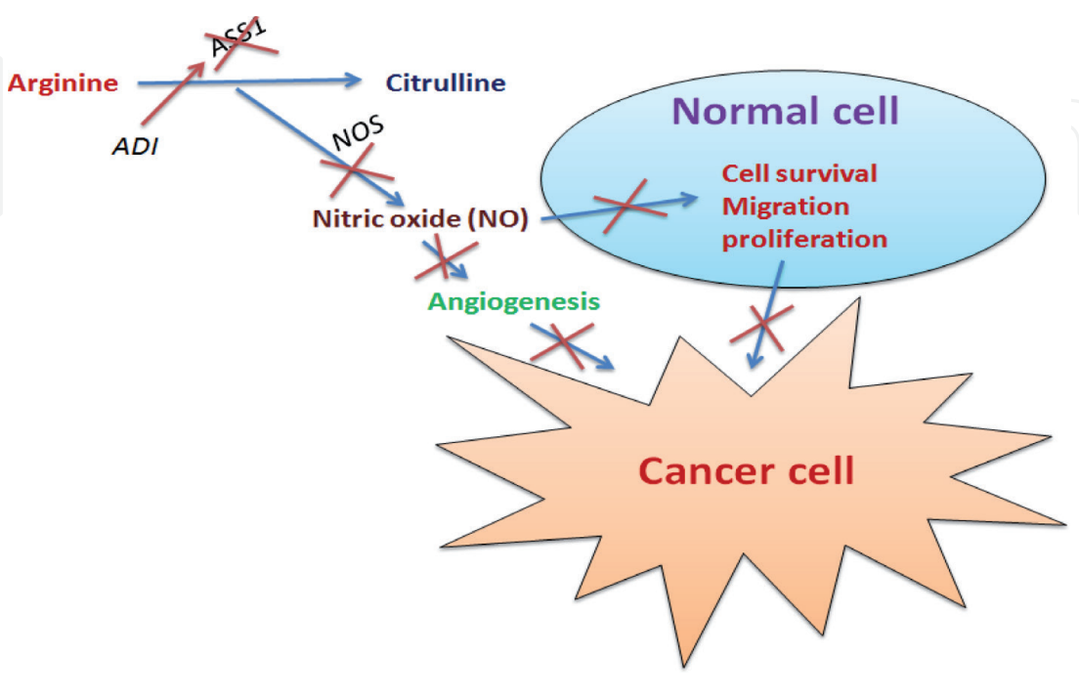

Figure 6.
Role of nitric oxide for angiogenesis and cancer proliferation and migration.

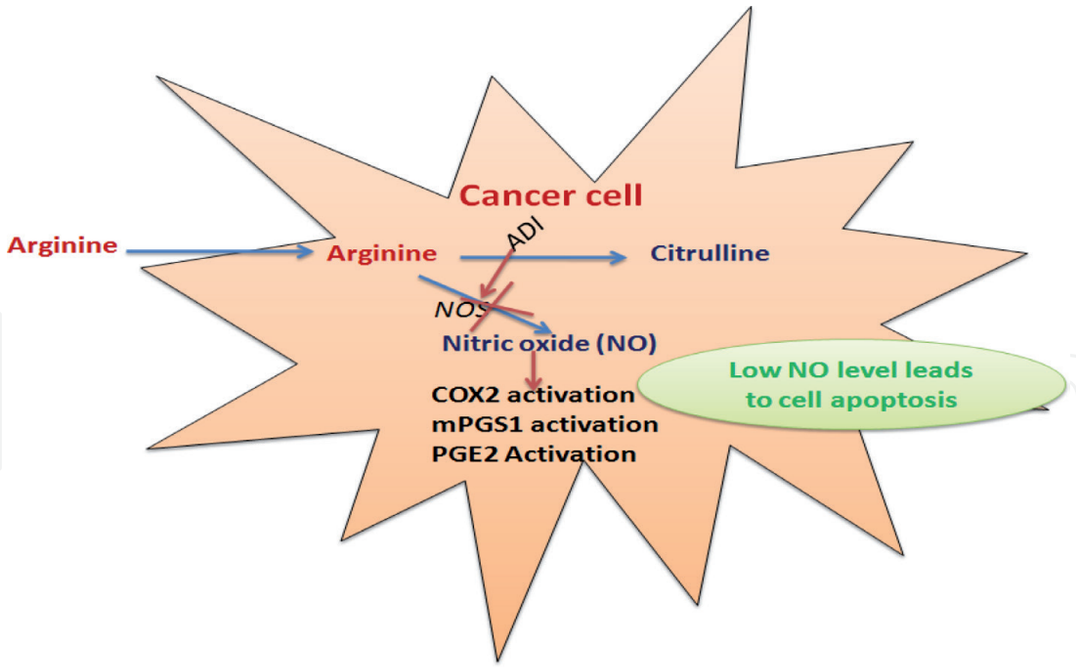

Figure 7.
Impact of ADI enzyme which leads the apoptosis in cancer cell.

mitochondrial inner membrane depolarization and Bcl-2-associated X protein (BAX) activation which is well known as program type II or caspase-independent cell death [55]. It was also reported that ASS1-deficient cancer cells, prolonged autophagy activated upon ADI treatment and impaired mitochondrial functions by inducing oxidative stress, chromatin autophagy and DNA leakage which finally causes cell death [61] as presented in **Figure 7**. In addition to this arginine limitation by pegylated (PEG) arginase causes cell death induced by necrosis as observed in acute myeloid leukemia (AML). In contrast, cell cycle arrest in AML cells did not induce cell apoptosis, autophagy, and rapid production of reactive oxygen species [62].

8. Role of arginine deprived agents

Deprivations of arginine from cancerous cells not only have a cytotoxic effect on cell but also induce specific cell cycle arrest. The cell cycle arrest analysis was done to check the surviving population of pancreatic and ovarian cancer cells to examine the consequence of arginine deprivation on cell cycle. The first reported arginine deprivation agent was ADI enzyme which degraded arginine and prevents cell growth s in culture from growing [63]. Human Arginase 1 (HuArgI) is second arginine deprivation agent and used to target arginine auxotrophic cancer cell lines and it is stable longer in serum, improved catalytic activity and less exposed with immune system [64]. Different types of cancerous cell lines undergo different mechanisms of cell death when deprived to arginine such as the process of autophagy, when cell degrades itself during nutrients limitation leads to starvation and cell death. Moreover, autophagy inhibited by HuArgI may indicate no caspase activation, no loss in membrane integrity and prevent the cell death caused by apoptosis [65].

9. In inhibition of cell migration

Cell migration is a well accepted attribute of the cancerous cell and arginine depletion majorly affect on cell viability and migration [66]. Low level of arginine

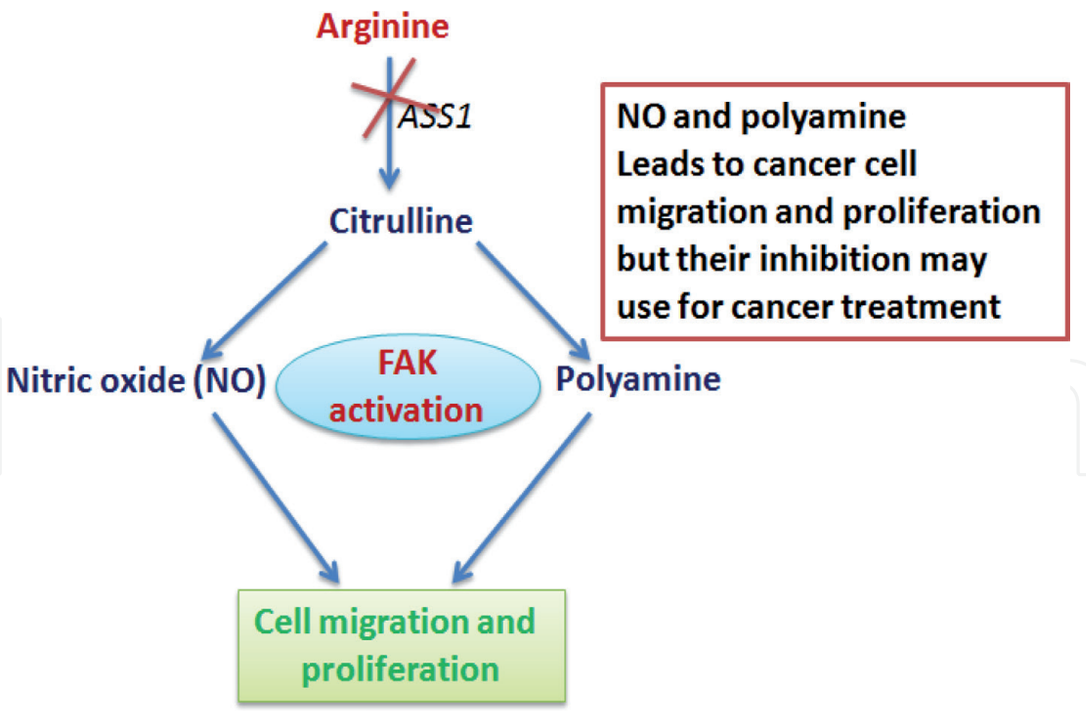

Figure 8.
Migration and proliferation of cancer.

accompany with the down-regulation of ASS1 expression which may leads the complete auxotrophic and mitigation of cancer cell migration [67]. The migration dependent on arginine requires optimum arginine to be catabolized two specific major enzymes nitric oxide synthase and arginase1 and produced citrulline and NO [68]. This increased level of promotes the cell viability, proliferation and migration during the process of wound healing [69]. Higher level of NO also activates signaling of focal adhesion kinase (FAK) cascade, which take parts in integrin assembly and disassembly as shown in **Figure 8**. Less arginine degradation to NO during wound healing showed a decrease in migration of colorectal cancer cells and added citrulline restored cell migration [70]. This higher nitric oxide synthase activity was recorded in intestinal epithelial cells in the presence of arginine and citrulline and NO production, which directly stimulate the cell migration [71]. The impact of arginine limitation and role of FAK was noticed when a study done on human intestinal epithelial cells which showed a significant role of NO production and cell migration [72]. Similar to this, other enzyme such as ornithine decarboxylase (ODC) used in polyamine biosynthesis also play important role in cell migration where polyamines increase the K^+ channel mediated Ca^{2+} influx and support to FAK activation [73]. The inhibition of ODC enzymes was majorly correlated with abnormal morphology of actin-cytoskeleton of metastatic cells migration [74]. Other signals including PI3K, Rho GTPases, microtubules and integrins always found to be interlinked and positive play important role in cell polarity by regulating intracellular junctions, cell adhesion, invasion and migration [75]. Arginine depletion also hamper the RhoA activation in colorectal cancer cells and during a report, the increased level of NO was majorly found to be involved in the RhoA activation in pancreatic cancer cells [76].

10. Conclusion

Several cancerous cells exhibited a higher metabolic requirement for specific amino acids to meet their rapid growth and migration. Therefore, specific amino

acid limitation could be a novel therapy target to cure cancer. Arginine is a well known essential amino acid with the ability to regulate cellular activities and influence viability, proliferation, motility, migration, adhesion and invasion. There are several clinical studies have been reported which clearly explain the impact of arginine limitation as a therapy to cure arginine auxotrophic tumors and arginine converted in to polyamines and NO, majorly focused for cell proliferation and migration. The role of ADI enzyme and less expression of ASS1 gene was found to be directly correlated with the production of NO and polyamines and elimination of arginine auxotrophic tumors. Some tumors such as hepatocellular carcinoma and melanoma are found to be very sensitive for this treatment of arginine limitation because here arginine does not take parts in the urea cycle. Thus, the development of a new drug and drug resistance due to induction of ASS1 expression leads to a potential problem in tumors curing. Overall, the complete mechanism understanding of arginine limitation and inhibition of arginine auxotrophic cancer cell proliferation and migration is not clear and still further investigation is required to understand this cancer therapy.

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