

## REVIEW ON ANTICANCER ENZYMES AND THEIR TARGETED AMINO ACIDS

Bhumi Prajapati and Naga Rathna Supriya \*

C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University.

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### \*Corresponding Author

**Dr. Naga Rathna Supriya**

C. G. Bhakta Institute of  
Biotechnology, Uka Tarsadia  
University.

### ABSTRACT

Cancer cells proliferate abnormally when compared to normal cells, as a result they require high amount of amino acids as nutrients because they are the building blocks for protein synthesis. Proteins are not only essential for the growth but also to form the structural basis of chromosomes. It clearly states that without amino acids, tumor cells fails to function because proteins can't be synthesized. Based on this concept recent research has targeted on amino acid metabolic enzymes that deregulate specific amino acid metabolism that is essential for cancer cell proliferation. These enzyme cuts off the supply of essential

amino acids which leads to nutrient starvation of cancer cells resulting in their death. Currently several amino acid metabolic enzymes are in clinical trials that specifically target amino acid metabolic pathways in tumor cells but not normal cells. L-Asparaginase, L-Glutaminase, L-Argininase, L-Methioninase are some of examples of enzymes that are used in cancer therapy that target specific amino acids resulting in disturbance of cancer cell proliferation without affecting normal cell metabolism. This review outlines different anticancer enzymes which effect on amino acids that disturb the metabolic pathway of cancer cells leading to its death simultaneously explaining different applications of enzymes as well as amino acids.

**KEYWORDS:** Cancer therapy, L-Asparaginase, L-Glutaminase, L-Argininase, L-methioninase.

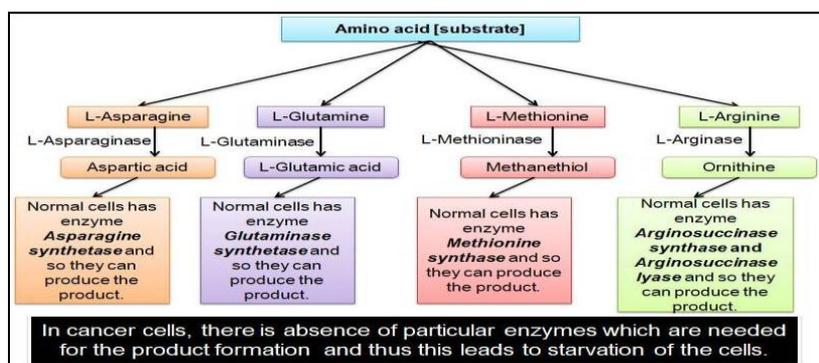
### INTRODUCTION

Amino acids play an important role in specific biological functions apart from protein synthesis. For example - Glutamine, Serine, Glycine, and Aspartate are needed for nucleotide biosynthesis.<sup>[1]</sup> Leucine, glutamine, and arginine serve as signaling molecules.<sup>[2]</sup> Previous

reports reveal that cancer cells require high amount of amino acids to support their fast proliferation rate. Enzymes targeting those amino acids required for the proliferation of cancerous cells are one of the tools used in cancer therapy now a day. Disrupting amino acids not only disturb DNA replication in cancer cells but also stops new blood vessels formation which stops the progression of cancer and helps to kill cancer cells.<sup>[3]</sup>

**Anticancer enzymes:** The application of enzyme technologies to pharmaceutical research, development and manufacturing is a growing field. The concept of therapeutic enzymes has been around for at least 50 years. For example, de Duve (1955) in the 1960s described a therapeutic enzyme as part of replacement therapies for genetic deficiencies.<sup>[5]</sup> Therapeutic enzymes have two main features; firstly that the enzymes act on their target with a great specificity and with high affinity, secondly they are catalytic and able to convert a substrate into a desired product.<sup>[4]</sup> These two features make enzymes specific and potent drugs that can accomplish therapeutic biochemistry in the body that small molecules cannot. These characteristics have resulted in the development of many enzyme drugs for a wide range of disorders. L-Asparaginase, L-Glutaminase, L-Argininase, L-methioninase are some of the examples of anticancer enzymes. L-Asparaginase breaks the L-asparagine, L-Glutaminase depletes L-Glutamine, L-Argininase cleaves L-Arginine, and L-Methioninase shows effect on L-methionine.

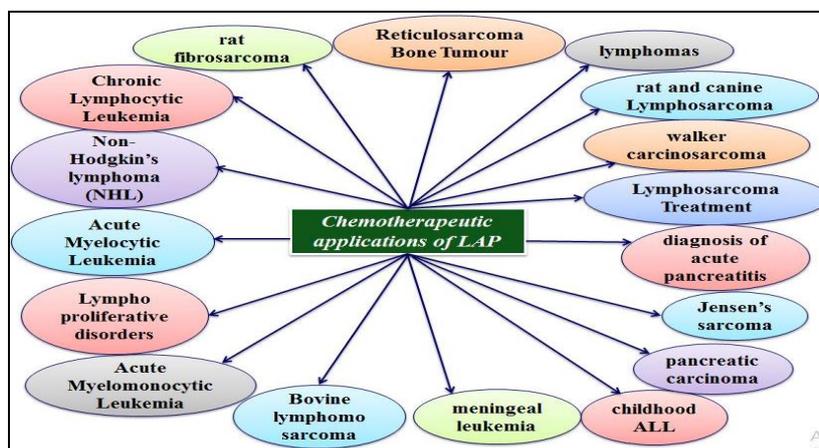
**Concept behind the anticancer nature of enzymes:** The key property of anticancer enzymes mentioned in this review is more or less similar. Anticancer enzymes catalyse specific amino acid and convert it into an unavailable form to the cells leading to starvation condition. The normal cell is not affected by this starvation condition because they have the ability to convert the product obtained by the action of anticancer enzyme into the required form for its growth.



**Fig. 1: Antineoplastic mechanism of anticancer enzymes.**

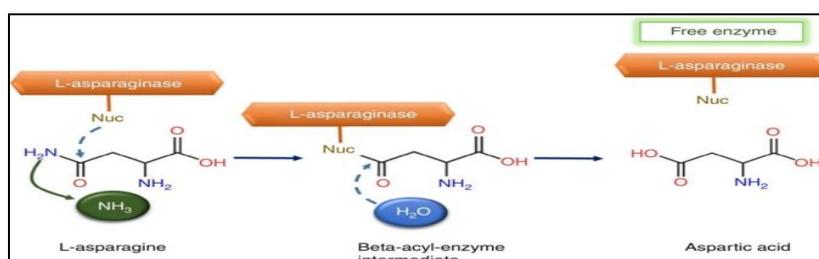
**L-Asparaginase (LAP):** L-Asparaginase (L-asparagine amino hydrolase, E.C. 3.5.1.1, LA) is an important enzyme used as chemotherapeutic agent for a treatment of human cancer and acts as a catalyst in the breakdown of asparagine to aspartic acid and ammonia.<sup>[6]</sup> Several brand name of L-asparaginase are available in the market such as *CLOLAR*, *ARRANON*, *LEUKINE*, *KIDROLASE*, *ONCASPAR*, *ELSPAR* and *ERWINASE*.

**a. Application of LAP:** L-asparaginase is used in the treatment of variety of lymphoproliferative disorders, lymphomas<sup>[7,8]</sup>, pancreatic carcinoma<sup>[9]</sup>, Bovine lymphosarcoma<sup>[10]</sup>, diagnosis of acute pancreatitis<sup>[11]</sup>, childhood ALL<sup>[12]</sup>, meningeal leukemia<sup>[13]</sup>, rat and canine Lymphosarcoma, rat fibrosarcoma, walker carcinosarcoma and Jensen's sarcoma<sup>[14,15]</sup>, acute myeloblastic leukemia (AML), Non-Hodgkin's lymphoma (NHL) and chronic leukemias (**Fig:2**).



**Fig. 2: Applications of LAP.**

**b. L-asparaginase Reactions:** The hydrolysis process occurs in two steps through an intermediate: beta-acyl-enzyme (**Fig.3**). In the first process step, the nucleophilic residue of the enzyme is activated by a strong base and attacks the amide carbon atom of L-asparagine (substrate), generating a product beta-acyl-enzyme intermediate. The second reaction step is an attack on the ester carbon made by a nucleophile activated by a water molecule.<sup>[8]</sup>



**Fig. 3: Catalytic reaction of LAP.**

Source: Based on Hill (1967), El-Bessoumy *et al.* (2004), and Shrivastava *et al.* (2016).<sup>[52,53,54]</sup>

### c. Mechanism of anticancer activity

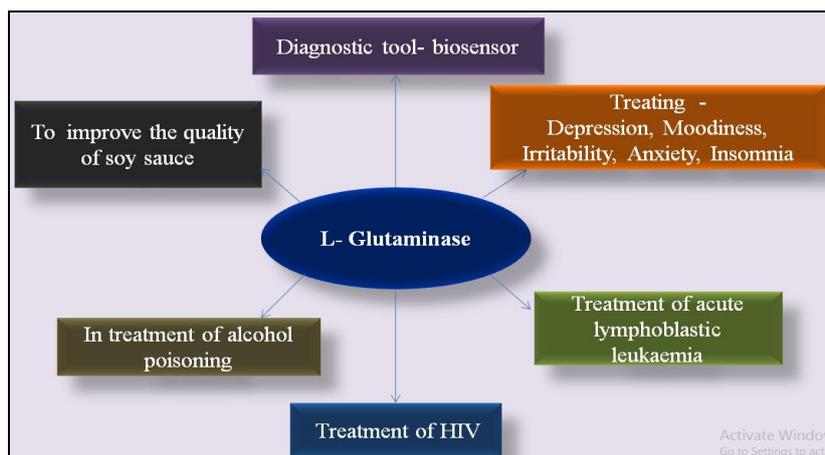
L-Asparagine is a key amino acid for RNA and Protein synthesis. It is required for proliferation and survival of tumor as well as normal cells.<sup>[18]</sup> Cancerous cells require high amount of L-Asparagine compared to the normal cell but it does not have the ability to produce L-asparagine, as a result it depends on serum L-asparagine for its survival.<sup>[82]</sup> Essential amino acid L-asparagine is hydrolyzed to aspartic acid and ammonia by LAP resulting in the unavailability of amino acid for the growth of tumor cell. Depletion of this amino acid does not affect the normal cells because they have the gene which codes for asparagine synthetase located on Chromosome number 7 (7q21.3) that converts aspartate to asparagine by using ATP as energy source.<sup>[18]</sup> As tumour cells lack L- asparagine synthetase gene they cannot produce L-asparagine resulting in starvation leading to apoptosis. Absence of asparagine results in arrest of cell cycle as shown in **Fig: 1**.<sup>[19,20]</sup>

## II. L-Glutaminase (GA)

L-Glutaminase (L-glutamine amidohydrolase E.C 3.5.1.2) is one of the anticancer enzymes that have been gaining importance now days.<sup>[23]</sup> It is not only useful in pharmaceutical industries but also in food industries. It has been reported that GA plays a major role in the nitrogen metabolism of both prokaryotes and eukaryotes. Gls and Gls2 are the two genes which are encoding GA.<sup>[24]</sup> L-Glutaminase Brand name which are available in the market are *Glutaminase F* “Amano 100”, *Flavopro*, *GLN*, *Glutaminase*, *Enzeco*. Based on the therapeutic and food industry use of L-glutaminase many researchers worked on the various aspects of L-glutaminase. Still a lot of research work is going on in the world wide.

### a. Applications of L-Glutaminase

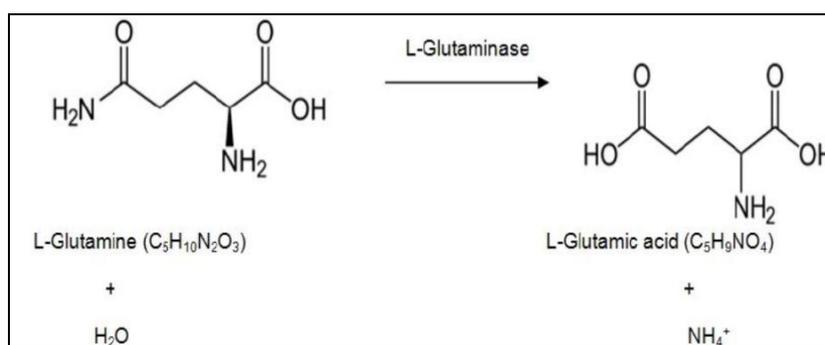
L-Glutaminase is not only used as an anticancer enzyme but it is also used for various purposes as mentioned in **(Fig:4)**. Diagnostic tool- biosensor, Treating Depression, Moodiness, Irritability, Anxiety, Insomnia, alcohol poisoning, acute lymphoblastic leukaemia, HIV, To improve the quality of soy sauce.<sup>[84]</sup>



**Fig. 4: Applications of Glutaminase.**

### b. L-Glutaminase reaction

GA converts L-glutamine to glutamic acid and ammonia which results in starvation of the tumor cell leading to apoptosis because they lack glutamine biosynthesis enzyme L-glutamine synthetase which is present in normal cell as shown in **(fig:5)**.<sup>[55]</sup>



**Fig. 5: catalytic reaction of GA.**

### c. Mechanism of action

Normal and Cancerous cells uptake necessary L-glutamine from the blood for their growth and survival. Requirement of L-glutamine is more for cancerous cell for its uncontrolled proliferation compared to normal cells. Some cancer cells show addiction to glutamine due to the oncogenic expression of the Myc gene, which codes for a transcription factor promoting expression of glutamine transporters and metabolic enzymes for biosynthesis.<sup>[24]</sup> When L-Glutaminase convert L-glutamine into glutamic acid, normal cell is not affected because normal cell gene has the capability of producing Glutaminase synthetase which convert glutamic acid to glutamine resulting in the normal functioning of cell metabolism.<sup>[25]</sup> In case of cancer cell Glutaminase synthetase enzyme is not found as a result the metabolisms where

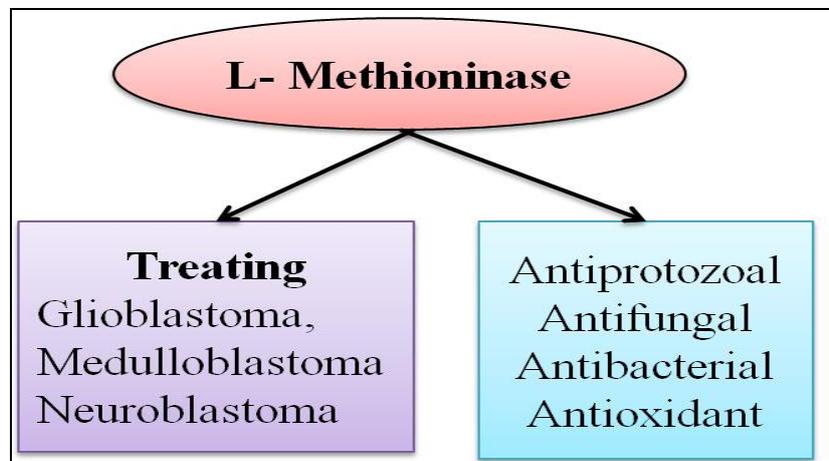
glutamine is required get disturbed. Scarcity of glutamine leads the cancerous cell to apoptosis. The mechanism of L-Glutaminase enzyme was shown in **Fig: 1**.

### III.-Methioninase (MGL)

The L-Methioninase has received affordable attention as a therapeutic agent against various types of methionine dependent tumors.<sup>[26]</sup> L-Methionine- $\gamma$ -lyase (EC 4.4.1.11; MGL) is one of the antitumor enzymes used in cancer therapy.<sup>[27]</sup> MGL is commonly known as methionase, methioninase, L-methionine- $\gamma$ -demethylase, L-methionine methanethiol -lyase (deamination). MGL is ubiquitous in all organisms except in mammals. MGL has a major role in food industry by imparting a distinctive aroma to many traditional fermented foods including cheese via degradation of L-methionine that releases volatile sulfur compounds (VSCs).<sup>[28]</sup>

#### a. Application of L-Methioninase

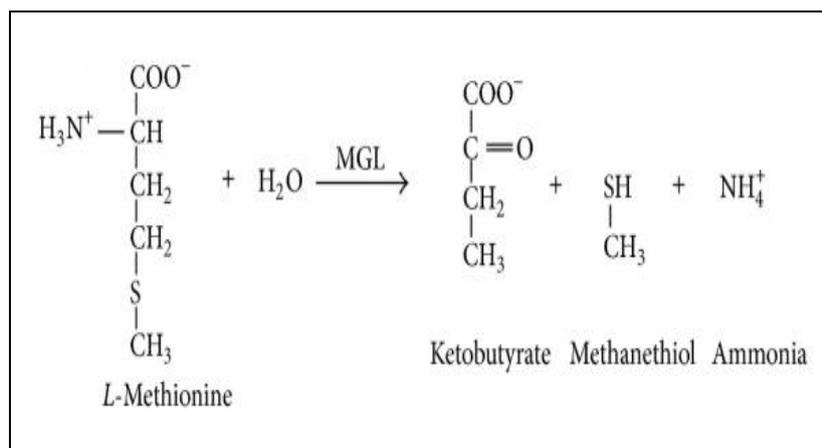
L-Methioninase was reported as a potent anticancer agent against various types of tumor cell lines: breast, lung, colon, kidney, and glioblastoma. It is also used as antiprotozoal, antifungal, antibacterial, antioxidant<sup>[29]</sup> as shown in (**fig.6**).



**Fig. 6: Application of L-Methionase.**

#### b. L- Methionase reaction

MGL catalyzes conversion of L-methionine to a ketobutyrate, methanethiol, and ammonia as shown in (**Fig.7**) by an  $\alpha,\gamma$  - elimination reaction.<sup>[31]</sup>



**Fig. 7: Catalytic reaction of L-Methionase.**<sup>[84]</sup>

### c. Mechanism of methioninase as anticancer drugs

Methionine plays a major role in many biological pathways as a result depletion of methionine may result in disturbance in the growth of the tumor cell. Methionine is substrate for S-adenosyl methionine (SAME), which is an essential methyl donor for numerous molecules within cells, methylation of DNA in the nucleus which decrease apoptosis, increase proliferation, and involves in tumor genesis.<sup>[37]</sup> Tumor cells require high amount of plasma methionine for their uncontrolled growth, whereas normal cells are relatively resistant to the restriction of exogenous methionine.<sup>[30]</sup> Due to methionine depletion the late S-G2 phase gets arrested in cancer cells leading to apoptosis.<sup>[36]</sup> Growth of normal cell will not be affected due to depletion of methionine as they can produce methionine synthase which helps in survival by utilizing homocysteine, instead of methionine.<sup>[38]</sup> Methionine synthase is not expressed in tumor cells; as a result they are subjected to starvation and eventually lead to apoptosis due to unavailability of external Methionine as shown in **(Fig:1)**.<sup>[39,40]</sup>

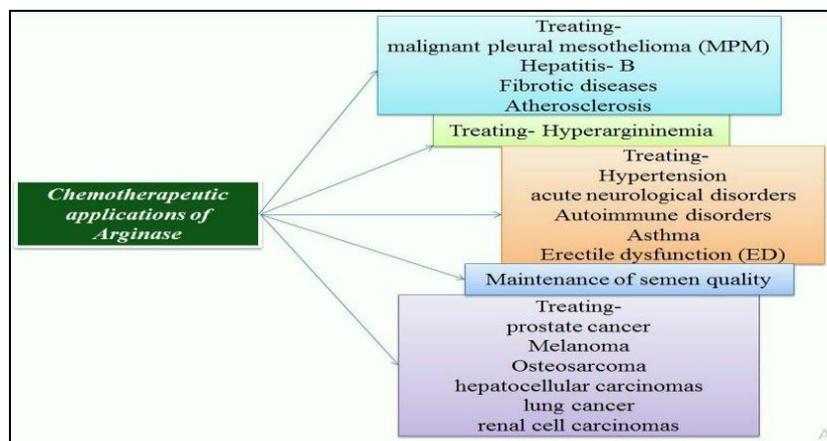
## IV. Arginase

Arginase has been used to treat arginine dependent cancers. Arginase (EC 3.5.3.1, *arginine amidinase, canavanase, L-arginase, arginine transamidinase*) is a manganese-containing enzyme that catalyzes the deamidation of L-arginine to L-ornithine and urea.<sup>[56,57]</sup>

### a. Applications of L-Arginase

Arginase affect the arginine auxotrophic cancers, such as melanoma, lung cancer, renal cell carcinomas and hepatocellular carcinomas<sup>[58,59,60,61,62,63,64,65]</sup>, acute neurological disorders<sup>[60]</sup>, regulator of penile and vaginal flow thus playing an important role in male and female sexual arousal<sup>[66]</sup>, neural regeneration pathways, maintenance of semen quality, treatment of

Hepatitis- B<sup>[67]</sup>, biosensors for monitoring arginine levels in juice samples<sup>[68]</sup>, prostate cancer,<sup>[69]</sup> human T-lymphoblastic leukemia,<sup>[70]</sup> and osteosarcoma.<sup>[62]</sup>, rheumatoid arthritis therapy<sup>[71]</sup>, an indirect regulator of penile and vaginal flow playing an important role in male and female sexual arousal<sup>[72]</sup> as shown in (Fig.8).

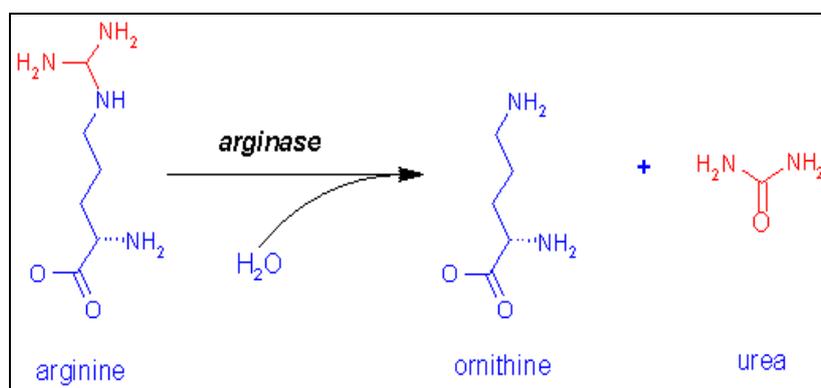


**Fig. 8: Applications of L-Arginase.**

### b. L-Arginase reaction

The Arginase catalyse the divalent cation-dependent hydrolysis of L-arginine to form the non-protein amino acid L-ornithine and urea as shown in (Fig.9).<sup>[73]</sup>

Urea -----urease-----> NH<sub>4</sub> + + CO<sub>2</sub>



**Fig. 9: Catalytic reaction of L-arginase.**

### c. Mechanism of Arginase as anticancer drugs

When Arginase converts L-Arginine to ornithine and urea the normal cells will not be effected because they have the ability to synthesize arginine from citrulline through the enzymes arginosuccinase synthase (AS) and arginosuccinase lyase, which is not expressed in Cancerous cells as shown in Fig:1.<sup>[83]</sup>

**Biological role of amino acids in normal cell**

Each amino acid has its own place in maintaining the normal metabolism. The common metabolic pathways that are interrupted due to the deficiency of amino acids mentioned in the paper are presented in table: 1. L-asparagine plays a vital role in glycoprotein biosynthesis, in maintaining nerve health, protecting liver, transportation of nitrogen, production of fatty acids, in normal functioning of the brain and central nervous system by bio transforming itself into required amino acid that helps in balancing the brain's chemicals that control excite or calm conditions in extreme measures. It is a precursor for some compounds necessary for the Krebs cycle.<sup>[22]</sup> Deficiency of Asparagine may suffer from poor metabolism and show inability to manufacture and excrete urea, resulting in depression, confusion, and headaches. L-Glutamine plays an important role in protein turnover, nutrient metabolism, immunity, acid-base balance, synthesis of nucleotides and hexosamines, energy production, redox homeostasis.<sup>[32, 33, 34]</sup> It helps in removing excess ammonia (which is a usual waste product), important for the digestion and for the normal brain function. It aids to protect the lining of the gastrointestinal tract and helps athletes to keep fit.<sup>[35]</sup> Methionine is precursor of L-Cysteine and L-Taurine which are important for any biological pathways.<sup>[41]</sup> It is useful in RNA synthesis. It is essential for production of polyamines (putrescine, spermidine, spermine).<sup>[42]</sup> It is crucial to maintain low homocysteine levels for the proper functioning of various pathways and for preventing the toxic effects of the cysteine homologue. Methionine acidifies the urine because it is directly or indirectly involved in sulphuric acid formation.<sup>[43]</sup> Methionine is important for many bodily functions, including immune cell production and proper nerve function.<sup>[44]</sup> Dietary methionine is an important amino acid for your liver's repair and rebuilding processes.<sup>[45]</sup> For pregnant women methionine, along with folic acid, plays a role in neural-tube (part of the fetus that forms during cell development and becomes the nervous system) defects in the fetus.<sup>[46,47]</sup> Methionine is an essential amino acid important for the normal closure of the neural tube, and research shows that methionine deficiency is associated with a higher occurrence of neural-tube defects (nervous system problems such as spina bifida) in newborns.<sup>[48,49]</sup> Methionine helps prevent skin and nail problems.<sup>[50]</sup> Methionine prevents excess fat buildup, helps relieve or prevent fatigue. Loss of methionine has been linked to senile greying of hair, L-Methionine is an intermediate in the biosynthesis of cysteine, carnitine, taurine, lecithin, phosphatidylcholine, and other phospholipids. Improper conversion of methionine can lead to atherosclerosis.<sup>[51]</sup> Arginine is involved in multiple pathways which are involved in major cellular functions such as nitric oxide production, creatine production.<sup>[74]</sup> Arginine is need for normal growth by increases the

release of the human growth hormone (HGH) (also known as the anti-aging hormone) from the pituitary gland<sup>[75]</sup>, maintenance of the body, precursor of nitric oxide, creatine, polyamines, agmatine and urea<sup>[76]</sup>, it is metabolically inter convertible with amino acids glutamate and proline, it decrease the incidence of gallstones<sup>[77]</sup>, Arginine being a metabolic precursor of nitric oxide an endogenous neurotransmitter helps to prevent vasoconstriction and which initiates vasodilation leading to increase blood circulation by relaxing the smooth muscle cells of the blood vessels.<sup>[78]</sup> Arginine increases stroke volume and cardiac output leading to increased blood circulation in congestive heart failure patients.<sup>[79]</sup> Arginine produces nitric oxide heals the colon ulcers by reducing intestinal permeability.<sup>[80]</sup> In interstitial cystitis condition arginine decrease pain and discomfort.<sup>[81]</sup> Arginine helps in curing male infertility as it involves in production spermidine which improves the sperm count and sperm motility as a result it is also known as the 'Natural Viagra' as it improves sperm count and sperm motility.<sup>[81]</sup> Low concentration of L-arginine leads to stress, illness, malnutrition or injury.

S. No	Metabolism	Required amino acid
1.	Cancer signaling, Gene Expression	L-Asparagine, L-Glutamine, L-Methionine, L-Arginine
2.	Cell growth	L-Asparagine, L-Glutamine, L-Methionine, L-Arginine
3.	Immunity	L-Asparagine, L-Glutamine
4.	Energy production	L-Asparagine, L-Glutamine
5.	Antioxidant	L-Glutamine, L-Methionine
6.	Polyamine synthesis	L-Methionine, L-Arginine

## OVERVIEW AND CONCLUSION

The main objective of this review article is to highlight the anticancer activity of anticancer enzymes based on amino acid starvation mechanism. From the above discussion it has been concluded that each enzyme has specific mechanism to catabolize the specific amino acid which results in the starvation of cancer cell but not normal cell as those amino acid can be produced with in them due to the expression of a particular enzyme which is unexpressed in tumor cells. This review has also high lightened different applications of anticancer enzymes apart from anticancer treatment. Even the specific amino acid normal role in the biological system has been mentioned giving an idea of how particular enzyme can affect different metabolisms in tumor cells.

## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication in this paper.

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