

Review Article

L-Methionase: A Therapeutic Enzyme to Treat Malignancies

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Cancer is an increasing cause of mortality and morbidity throughout the world. *L*-methionase has potential application against many types of cancers. *L*-Methionase is an intracellular enzyme in bacterial species, an extracellular enzyme in fungi, and absent in mammals. *L*-Methionase producing bacterial strain(s) can be isolated by 5,5'-dithio-bis-(2-nitrobenzoic acid) as a screening dye. *L*-Methionine plays an important role in tumour cells. These cells become methionine dependent and eventually follow apoptosis due to methionine limitation in cancer cells. *L*-Methionine also plays an indispensable role in gene activation and inactivation due to hypermethylation and/or hypomethylation. Membrane transporters such as GLUT1 and ion channels like Na^{2+} , Ca^{2+} , K^{+} , and Cl^{-} become overexpressed. Further, the α -subunit of ATP synthase plays a role in cancer cells growth and development by providing them enhanced nutritional requirements. Currently, selenomethionine is also used as a prodrug in cancer therapy along with enzyme methionase that converts prodrug into active toxic chemical(s) that causes death of cancerous cells/tissue. More recently, fusion protein (FP) consisting of *L*-methionase linked to annexin-V has been used in cancer therapy. The fusion proteins have advantage that they have specificity only for cancer cells and do not harm the normal cells.

1. Introduction

L-Methionine- γ -lyase (EC 4.4.1.11; MGL), also known as methionase, methioninase, *L*-methionine- γ -demethylase, and *L*-methionine methanethiol-lyase (deaminating), is a pyridoxal phosphate (PLP) dependent enzyme. PLP reduces the energy for conversion of amino acids to a zwitterionic carbonion [1] and substantially the apoenzyme catalyzes the cleavage of substrate bond yielding the product [2]. MGL is a cytosolic enzyme inducibly formed by addition of *L*-methionine to the culture medium [3]. MGL has a molecular weight (Mr) of about 149 kDa to 173 kDa and consists of four subunits with identical Mr of about 41 kDa to 45 kDa each except MGL purified to homogeneity from *Pseudomonas putida* (*ovalis*) which was found to consist of two nonidentical subunits of 40 kDa and 48 kDa [4].

L-Methionine must be incorporated into the human diet in order to biosynthesize *L*-cysteine (Figure 1) by *trans*-sulfuration pathway [5]. In yeast, methionine and cysteine

supplementation was required in order to biosynthesize cysteine or methionine, respectively. The microorganisms can synthesize the sulphur containing amino acids by utilizing inorganic sulphate via the *de novo* cysteine biosynthesis pathway [6]. *Escherichia coli* and plants utilize the forward *trans*-sulfuration pathway such that methionine is biosynthesized from cysteine or they may utilize inorganic sulphate via *de novo* cysteine biosynthesis [7, 8]. There are different kinds of methionine biosynthesis pathways in different organisms as described in the MetaCyc database. *E. coli* K-12 methionine biosynthesis-I pathway that involves methionine biosynthesis from homoserine, methionine biosynthesis by transsulfuration. *Arabidopsis thaliana*, methionine biosynthesis-II pathway that involves methionine biosynthesis from homoserine-II. *Corynebacterium glutamicum*, *Leptospira meyeri*, and *Saccharomyces cerevisiae* follow methionine biosynthesis-III pathway that performs homoserine methionine biosynthesis and methionine biosynthesis by sulfhydration. *Arabidopsis thaliana*,

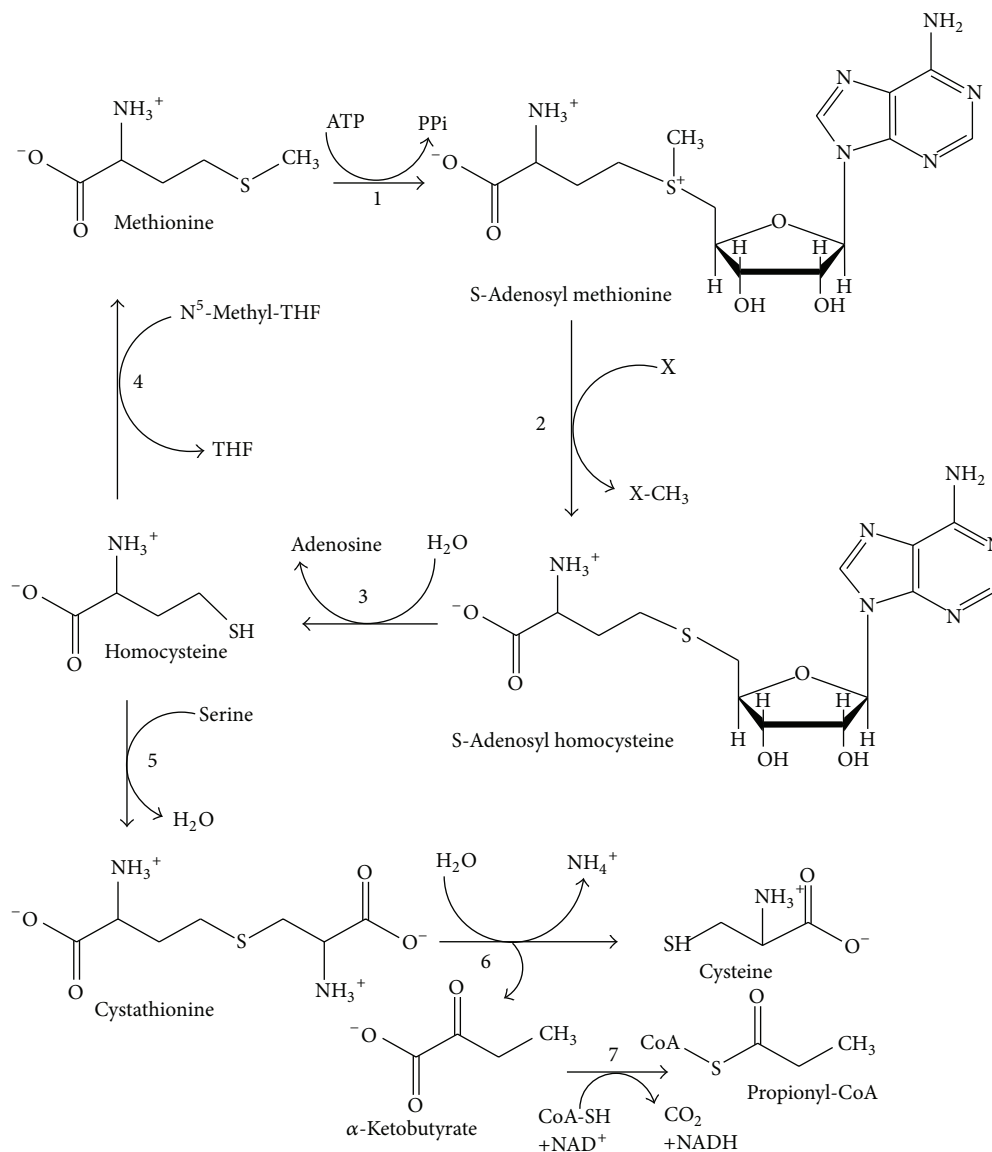


FIGURE 1: General approach of methionine metabolism (modified from [46]). *L*-Methionine is mainly supplied exogenously from dietary proteins. The enzymes involved in this pathway are (1) methionine adenosyltransferase; (2) *S*-adenosylmethionine methyltransferases; (3) adenosylhomocysteinase; (4) 5-methyltetrahydrofolate-homocysteine methyltransferase (in mammals betaine-homocysteine methyltransferase or homocysteine methyltransferase); (5) cystathionine- γ -synthase; (6) cystathionine- γ -lyase; (7) α -ketoacid dehydrogenase.

Bacillus subtilis, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lupinus luteus*, and *Oryza sativa* follow methionine salvage-I pathway while *Homo sapiens* and *Rattus norvegicus* possess methionine salvage-II system. *Bacillus subtilis*, *Corynebacterium glutamicum*, *Leptospira meyeri*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Saccharomyces cerevisiae* possess a unique superpathway of methionine biosynthesis (by sulfhydrylation). On other hand, *Arabidopsis thaliana*, *Lupinus luteus*, *Oryza sativa*, *Plantago major*, and *Solanum lycopersicum* follow Yang cycle/MTA cycle [9]. *E. histolytica* and *T. vaginalis* have a methionine catabolic pathway and elements of a *de novo* sulphide biosynthetic pathway for cysteine biosynthesis in *E. histolytica*. These differences in

cysteine metabolism between humans and parasites are of particular interest, especially for the future development of antiparasitic compounds. Currently, *de novo* engineering of a human MGL has been followed for achieving systemic *L*-methionine depletion in cancer therapy [10].

2. Sources of MGL

MGL is widely distributed in bacteria, especially in *Pseudomonas* spp. and is induced by the addition of *L*-methionine to the culture medium. Crystal structures of MGL have been reported from *Pseudomonas putida* (*P. putida*) [17, 18], *Citrobacter freundii* [47], *Trichomonas vaginalis* [3],

TABLE 1: Potential sources for *L*-methionine γ -lyase isolation.

Source	Examples	Reference(s)
Bacteria	<i>Brevibacterium linens</i>	[11]
	<i>Clostridium sporogenes</i>	[12]
	<i>Citrobacter intermedius</i>	[13]
	<i>Citrobacter freundii</i>	[14]
	<i>Porphyromonas gingivalis</i>	[15]
	<i>Pseudomonas ovalis</i>	[16]
	<i>Pseudomonas putida</i>	[17, 18]
	<i>Treponema denticola</i>	[19]
	<i>Micrococcus luteus</i> , <i>Arthrobacter</i> sp., <i>Corynebacterium glutamicum</i> and <i>Staphylococcus equorum</i>	[20]
Protozoa	<i>Trichomonas vaginalis</i>	[3]
	<i>Entamoeba histolytica</i>	[21–23]
Plant	<i>Arabidopsis thaliana</i>	[24]
Archaeon	<i>Ferroplasma acidarmanus</i>	[25]
	<i>Aspergillus</i> sp. RS-1a	[26]
Fungus	<i>Geotrichum candidum</i>	[27]
	<i>Aspergillus flavipes</i>	
	<i>Scopulariopsis brevicaulis</i>	
	<i>A. carneus</i>	
	<i>Penicillium notatum</i>	[28]
	<i>Fusarium solani</i>	

and *Entamoeba histolytica* [21, 22]. The MGL was isolated from different sources such as bacterial, protozoans, fungal, archaeon, and plants (Table 1).

3. MGL Isolation

The bacterial, protozoans, archaeal, and plants produce intracellular MGL and fungal sources produce extracellular MGL. Therefore isolation of MGL from microbial sources required cell disruption by chemical, enzymatic, and mechanical methods. Fungal sources produce extracellular MGL; thus, there is no need for cell disruption. Amongst above described MGL sources, *P. putida* is reported to be the best source for MGL production. *P. putida* cell pellets were disrupted by passage through French press. The ammonium sulphate precipitated crude cell lysate was applied on DEAE-cellulose and Sephadex G200 column, respectively. The MGL specific activity (Units/mg protein) from *P. putida* was 14.20 that improved to 3,735 after column chromatography, whereas, in case of *Aspergillus flavipes*, the specific activity (Units/mg protein) was 12.58. *A. flavipes* required 10-day incubation period for growth and 8 days for production time, whereas *P. putida* needed 24–48 h incubation and production time for growth [17, 28]. Microorganisms are most important and convenient sources of commercial enzymes production. Moreover, they have an advantage that they can be cultivated by using inexpensive media and enzyme production occurs in short time.

4. Biochemical Reaction Catalyzed by MGL

MGL catalyzes the conversion of *L*-methionine to α -keto-butyrate, methanethiol, and ammonia by α , γ -elimination reaction (Figure 2).

5. Methionase Assay

The free sulphhydryl group in solution could be quantitatively measured [48] by 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB). The DTNB was used as screening dye in agar media to detect methanethiol, which reduces DTNB to form yellow coloured aryl mercaptan (2-nitro-5-thiobenzoate or TNB) around the bacterial colony that is able to produce MGL enzyme. DTNB has little absorbance, but when it reacts with thiol (SH) groups on proteins under mild alkaline conditions (pH 7-8), the 2-nitro-5-thiobenzoate anion (TNB²⁻) gives an intense yellow color (Figure 3). Ellman's reagent is useful assay reagent because of its specificity for SH groups [49] at neutral pH, high molar extinction coefficient, and short reaction time. MGL activity was quantitatively assayed by 3-methyl-2-benzothiazolone hydrazone (MBTH) which determines the amount of α -ketobutyrate produced spectrophotometrically at 320 nm. The 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyl-tetrazolium bromide (MTT) assay [50] was used to determine the *in vitro* growth inhibition of tumour cells by MGL treatment.

6. Methionine Requirement in Cancer Cell

Tumours cells have uncontrolled rapid growth and proliferation as compared to the normal cells [51]. Many malignant human cell lines have enhanced requirements of methionine for high protein synthesis and regulation of DNA expression in cancer cells [31, 52–57]. Methionine is converted to S-adenosylmethionine and it becomes methyl donor for DNA methylation, an epigenetic phenomenon [58–61] associated with cancer (Figure 4). The high methionine diets were associated with increased prostate cancer risk. The higher availability of *L*-methionine leads to higher bioavailability of S-adenosylmethionine to donate methyl groups to DNA, resulting in DNA hypermethylation of regulatory regions, including tumour suppressors [62, 63].

The CpG is a cytosine-guanosine (CG) dinucleotide DNA sequence, in which the cytosine undergoes chemical modification to contain a methyl group. The methyl binding protein (MBP) primarily was involved in gene regulation of normal cells to exert transcriptional control and also exploited by cancer cells to escape such control [64]. DNA methylation is essential for normal development but in some diseases, such as cancer, gene promoter CpG islands acquire abnormal hypermethylation. The transcriptional silencing due to hypermethylation was inherited by daughter cells following cell division. Alterations of DNA methylation have been recognized to play important role in cancer development. The CpG hypermethylation has been observed in cancer cell lines such as breast, colon, lung, head and neck squamous cell carcinomas, glioblastoma, acute myeloid leukemia,

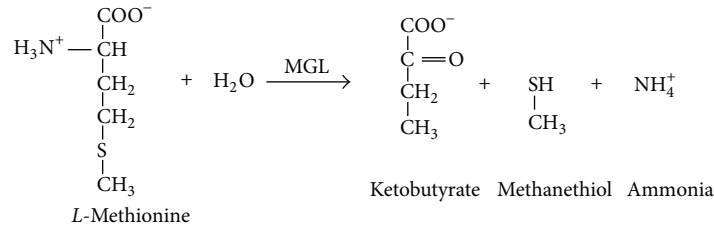


FIGURE 2: The biochemical reaction catalysed by MGL. MGL catalyses conversion of *L*-methionine into α -ketobutyrate, methanethiol, and ammonia by α, γ elimination reaction.

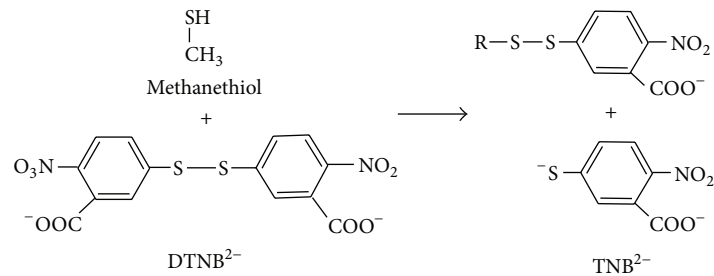


FIGURE 3: Methionase qualitative detection by DTNB. Methionase catalyses conversion of *L*-methionine as a substrate in agar plates into α -ketobutyrate, ammonia, and methanethiol. DTNB reagent reacts with SH (thiol) functional group and gives intense yellow coloration around methionase producing bacterial isolates.

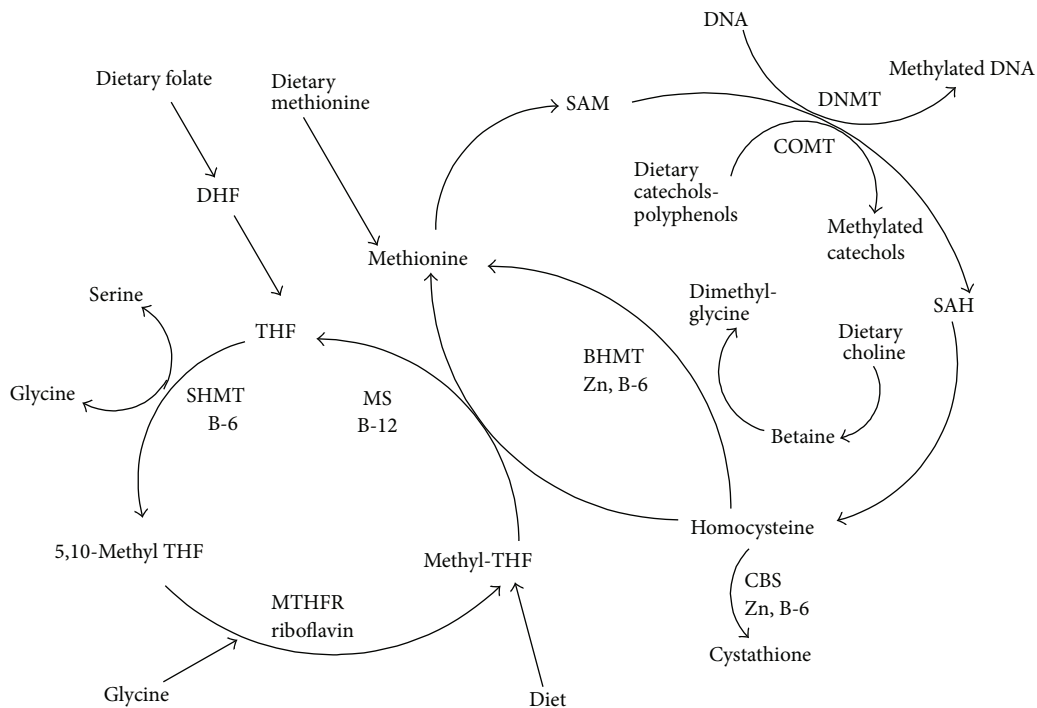


FIGURE 4: The pathways involved in cellular metabolism and production of S-adenosylmethionine (SAM) for DNA methylation [61]. Dietary factors regulate DNA and histone methylation such as BHMT betaine-homocysteine methyltransferase; CBS cystathionine β -synthase; COMT catechol-O-methyltransferase; DHF dihydrofolate; MS methionine synthase; MTHFR 5, 10-methylenetetrahydrofolate reductase; SAH S-adenosyl homocysteine; SAM S-adenosyl methionine; SHMT serine hydroxymethyltransferase and THF tetrahydrofolate.

medulloblastoma, and testicular germ cells tumours [65]. UHRF1 (also known as ICBP90) and DNA methyltransferases (DNMTs) are involved in maintenance of mammalian DNA methylation. UHRF1 (ubiquitin-like, two zinc-finger domains PHD, RING), also known as NP95 in mouse and ICBP90 in human, is required for maintaining DNA methylation. ICBP90 binds to the methylated retinoblastoma gene (RB1) gene promoter in the G1 phase and allows cells to smoothly enter the S phase [66, 67]. DNMT1 was found to be the sole detectable DNA methyltransferase in all murine tissues and cell types examined till date. Pyrosequencing assays were used to measure the DNA methylation of CDKN2A, RASSF1, CYGB, CDH13, DNMT1, DNMT3A, DNMT3B, and UHRF1 promoters [68]. UHRF1 overexpression in zebrafish hepatocytes causes DNA hypomethylation, Tp53-mediated senescence, and hepatocellular carcinoma [69, 70]. Restriction landmark genomic scanning (RLGS) was also used to assess the methylation in human malignancies. *L*-Methionine downregulates the genes belonging to protein kinase families on MCF-7 breast cancer cells and LNCaP prostate cancer cells and showed antiproliferative effect. *L*-Methionine also activates some of the genes involved in cellular redox regulation [71]. *L*-Methionine is required for the biosynthesis of the polyamines spermine and spermidine, which are mainly involved in cell proliferation [72]. The site-specific hypermethylation of cancer-related genes and miRNAs (microRNAs) hypomethylation occur in many cancers. Hypomethylation of miRNAs result in genome instability and activation of protooncogenes. The hypermethylation causes repression of tumour suppressor miRNAs by hypermethylation of their corresponding promoter loci. The miRNAs regulate gene expression within a cell and in the neighboring cells [73–75].

The normal cells have methionine synthase and can form methionine from homocysteine by methyl tetrahydrofolate and betaine as methyl group donors [76]. Methionine-dependent tumour cell lines present no or low levels of methionine synthase [51]. The dependence of tumours on methionine synthase for various cell lines in comparison to the normal cells has been previously reported [77–79]. *L*-Methionine is required for the synthesis of vitamins, antioxidants, DNA stabilizers, epigenetic DNA modulators, coenzymes [61, 80, 81], proteins, polyamines (proper cell development), antioxidative stress defense (glutathione/trypanothione), iron-sulfur cluster biosynthesis (energy metabolism), and methylation reactions and it also regulates the gene expression [5–8, 78]. The *L*-methionine is the first amino acid incorporated into many functional proteins during translation and also serves as a precursor for cysteine biosynthesis. Methionine dependence has been observed in many human cancer cell lines and cancer xenografts in animal models [82–84]. Methionine dependence is a metabolic defect seen only in cancer cells and such malignant cells do not grow in a medium in which methionine is depleted [30, 85]. Thus, *L*-methionase has received appreciable attention as a therapeutic agent against various types of methionine dependent tumours [86]. Dietary factors and epigenetic regulator play essential roles in anti-tumour activities [87]. Several approaches such as starvation of the tumour cells for methionine using methionine-free

TABLE 2: Cancer cells lines that possess methionine dependency.

Methionine dependent cell line(s)	References
PC-3 cell line human prostate	[29]
Prostate cancer PC3; lung carcinoma SKLU-I; fibrosarcoma HT 1080	[30]
Lung adenocarcinoma A-549 and the acute lymphoblastic leukemia CCRF-HSB-2,	[31]
W 256	[32]
D-54, SWB77 (human glioblastomas) and Daoy (human medulloblastoma)	[33]
Human melanoma cell line MeWoLC1	[34]

diets display a reliable efficacy against various types of tumour cells [88]. When tumour cells were deprived of methionine in a homocysteine containing medium *in vitro*, they were reversibly arrested in the late S/G₂ phase of the cell cycle and finally undergo apoptosis [89–91]. The methionine/valine depleted, tyrosine lowered, and arginine enriched in the diet were the most rationalized form of diet to achieve inhibition of tumour growth [92, 93]. The methionine-free diet is therapeutically not efficient due to economic and technical considerations [88]. A breast cancer cell line MDAMB468 showed methionine dependence and this dependency was due to SAM limitation [94]. There are a few other methionine dependent cell lines (Table 2) reported in the literature.

Cancer cells showed Warburg effect that refers to an increased utilization of glucose via glycolysis and was common in cancerous cells [95]. Glucose transport in cells is rate-limiting step for glucose metabolism mediated by facilitative glucose transporter (GLUT) proteins. The sugar transporters become activated in cancer cells so they incorporate higher amounts of sugar than normal cells. In tumour cells membrane transporter and channel proteins enhance uptake from outer sources and endogenous synthesis increases amongst many transporters glucose transporters (GLUTs) and sodium dependent sugar transporters (SGLT) play main role [96, 97]. The SGLT transporters comprises the sodium-glucose symporter SGLT2 expression was significantly higher in liver and lymph node [98]. The tumour has increased fatty acid synthesis and increased rate(s) of glutamine metabolism. High degree of GLUT1 expression has been reported in human hepatocellular carcinoma, oral cancer [99] and human pancreatic carcinoma (PC) cell line [100–105], and MKN45 (human gastric cancer). The glucose passes through membrane by facilitated diffusion via GLUT or by active transport through a SGLT [106]. Therefore cellular metabolic enzymes such as glucose transporters, hexokinase, pyruvate kinase, lactate dehydrogenase, pyruvate dehydrogenase kinase, fatty acid synthase, and glutaminase targeting enhance the efficacy of common therapeutic agents [95]. GLUT-1 overexpression increased matrix metalloproteinase-2 (MMP-2) promoter activity and was involved in binding of p53 to the MMP-2 promoter [107]. Solute-linked carrier family A1 member 5 (SLC1A5) mediates glutamine transport was overexpressed an associated with squamous lung cancer

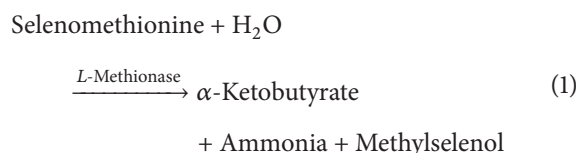
[108]. CPT-1 transporter helps in fatty acids transport in the form of acyl CoA and converted acetyl CoA. Acetyl CoA enters the TCA cycle and produces NADH which fuels the cell by oxidative phosphorylation [104]. AKT (protein kinase B PKB), a serine/threonine specific protein kinase activation, promotes cell growth, survival, and upregulation of ER-UDP hydrolysis enzyme as observed in human cancers. The ectonucleoside triphosphate diphosphohydrolase 5 (ENTPD5), an endoplasmic reticulum (ER) enzyme, elevated lactate production under aerobic conditions [109]. ENTPD5 expression and AKT activation is common in both cultured prostate cancer cell lines and primary human prostate carcinoma. Lowered ATP/AMP ratio increases glycolysis, elevates lactate production, and provides glycolytic intermediates for biomass production. The overexpressed α -subunit of ATP synthase, in breast cancer, was involved in the progression and metastasis of breast cancer [110, 111]. Periplocin downregulated the ATP synthase ecto- α -subunit (ATP5A1) and eukaryotic translation initiation factor 5A-1 (eIF5A) by periplocin mediated growth inhibition of A549 cells [112]. ATP synthase was upregulated in cancer cells [113, 114]. Ion channels like Na^{2+} , Ca^{2+} , K^{+} , and Cl^{-} play significant role in cells. The intracellular chloride channel (CLIC) plays an essential role in cellular function, pH, electrogenic balance and maintaining membrane potential in organelles. The chloride channel (CLIC1-5) except CLIC4 became overexpressed in cancer cells. CLIC4 expression reduced in tumour cells [115, 116] and ion channels used to inhibit cancer cell growth [117]. The flow of potassium ions plays important functions, such as cell proliferation, angiogenesis or cell migration, which have also recently been assessed [118, 119]. ABC transporters require energy in the form of adenosine triphosphate (ATP) to translocate substrates across cell membranes. This protein can transport cationic or electrically neutral substrates as well as a broad spectrum of amphiphilic substrates [120]. The ABCG2 (G-subfamily of human ABC) transporter was downregulated in cancer cells [121]. ABC transporters showed multidrug resistance (MDR) in cancer cells by the overexpression of ABC transporters which increased efflux of drugs from cancer cells, thereby decreasing intracellular drug concentration [122, 123].

7. Utilization of MGL in Cancer Therapy

7.1. Combinational Therapy. Therapeutic exploitation of *P. putida* MGL to deplete plasma methionine has been extensively investigated [65, 66]. The MGL was tested as a potent antiproliferative enzyme towards Lewis lung and human colon carcinoma [124], glioblastoma [33], and neuroblastoma [125]. The cancer cell targeted drugs, that is, small molecules, are not fully effective because cancer stem cells are able to expel the drugs before the cancer cells are destroyed and the cancer cells are then able to renew and produce relapse of the disease. A therapeutic approach to deplete methionine from tumours is to treat the cells with recombinant MGL from *P. putida* [PpMGL]. The growth of human tumours *in vivo* and *in vitro* (xenografted in nude mice) is

reported to be inhibited upon treatment with recombinant PpMGL when compared to normal cells [126]. Reduction in cell growth is also achieved by integrating PpMGL gene into human lung cancer cells by using a retroviral-based vector. The treatment with exogenous recombinant PpMGL, in order to deplete intracellular and extracellular methionine (Figure 5) levels, has been attempted [38, 127]. MGL alone or in combination with chemotherapeutic agents such as cisplatin, 5-fluorouracil (5-FU), 1-3-bis(2-chloroethyl)-1-nitrosourea (BCNU), and vincristine has shown efficacy and synergy, respectively, in mouse models of colon cancer, lung cancer, and brain cancer [41, 125, 128]. It was also reported that MGL introduced by adenovirus vector inhibited the growth of tumours *in vitro*. MGL, when combined with selenomethionine [SeMET], a suicide prodrug substrate of MGL, inhibited tumour growth in rodents and prolonged their survivals [127].

The effect of prodrug [Selenomethionine] and the toxic product [Methylselenol] synthesized in the tumour cells is presented below:



The MGL gene product, α -methionine- γ -lyase converts nontoxic SeMET to methylselenol that catalyzes oxidation of thiols to generate toxic superoxide. Apoptosis occurs mainly via a mitochondrial pathway [89]. Methylselenol readily diffused to the surrounding nontransduced tumour cells, destroying the mitochondrial membrane by the oxidative stress [39]. Treatment of the transduced cells with exogenous selenomethionine is found to inhibit tumour cell growth [129]. The methylselenol is required in very low concentration to induce cell cycle arrest and apoptosis [130]. Methylselenol promotes the expression of matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) that inhibits the migration of tumour cells [131]. Methylselenol induced apoptosis reported in many cancer cells such as murine melanoma B16F10 [132], fibrosarcoma cells HT1080 [130, 131, 133], colon cancer derived HCT-116 [134], and human prostate cancer cells LNCaP [135]. Methylselenol inhibits cell proliferation in the cancerous HCT116 cells as compared to normal cells NCM460 [134]. Methylselenol rapidly decreased cellular prostate-specific antigen (PSA) level in LNCaP cells [135]. ROS promote cell proliferation in low concentration, whereas increase of ROS can induce cell death. Therefore balance between generation and elimination of ROS maintains the proper function. Methylselenol catalyzes the oxidation of thiols, generating toxic ROS causing mitochondrial swelling, releasing cytochrome C, activating the caspase cascade, and inducing the cell apoptosis and death [136]. Selenomethionine is relatively nontoxic to the mammalian cells due to their lack of L-methionase. The maximum antiproliferative activity was observed by selenomethionine methionase treatment [137]. The sensitivity of tumour cells to selenomethionine was increased by 1,000-fold via transduction by adenovirus mediated methionase gene [138]. The combination

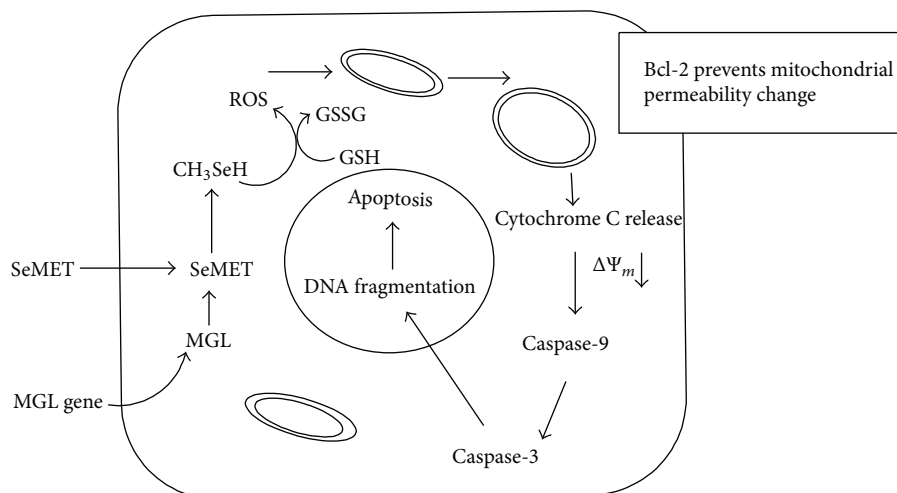


FIGURE 5: A proposed mechanism of MGL/SeMET-induced apoptosis by combinational therapy method. MGL gene (PpMGL) along with selenomethionine as prodrug was inserted inside the tumour cells. MGL gene product *L*-methionase catalyses the breakdown of methionine from prodrug and synthesizes a toxic molecule “methylselenol” that produces reactive oxygen species (ROS). The ROS thereby causes mitochondrial apoptosis by caspase activation.

of methionase gene, methionase, and selenomethionine are effective against all methionine dependent tumours [39, 127].

7.2. Use of Fusion Proteins in Cancer Cell Targeting. The oxidative stress in tumour cells caused exposure of phosphatidylserine on the surface of the vascular endothelium of blood vessels in tumours but not on normal cells [139]. The fusion protein (FP) consisting of *L*-methionase linked to human annexin-V injected into the bloodstream will bind to the marker on vascular endothelial cells of the tumour only. The FP catalyzed the conversion of nontoxic prodrug selenomethionine into toxic methylselenol and also prevented the methionine supplementation to the tumour cells, thereby killing the tumour and/or inhibiting its growth due to methionine restriction [140–142]. The great advantage of FP is that it does not require to be delivered directly to the tumour cells but only to the bloodstream. ATF-methionase FP (amino-terminal fragment of urokinase) was used to inhibit cancer cell proliferation and migration, which supports targeting *L*-methionase to the surface of the cancer cells. The FP has potential as a selective therapeutic agent for the treatment of various methionine-dependent cancers [143].

8. Modifications of *L*-Methionase to Reduce Its Side Effects

Tumour growth inhibitory effect of rMGL and PEG-MGL on human cancer cells such as human lung, colon, kidney, brain, prostate, and melanoma cancer cells and lung cancer orthotopic model [38, 144]. It was reported that administration of MGL resulted in a steady-state depletion of plasma methionine to less than 2 μ M concentration. The

only manifested toxicity was a decreased food intake and a slight weight loss. Serum albumin and red cell values declined transiently during treatment, which might be related to extensive blood sampling, although vomiting was frequently observed in macaque monkeys [145]. To overcome this problem, polyethylene glycol-conjugated MGL (PEG-MGL) was prepared. Simultaneous coadministration of pyridoxal 5'-phosphate and oleic acid or dithiothreitol treatment also strengthened effectiveness of PEG-MGL. To improve the MGL therapeutic potential, MGL was coupled to methoxy polyethylene glycol succinimidyl glutarate-5000 (MEGC-PEG-5000). The half-life due to pegylation increased 6 to 19 times while plasma methionine depletion efficacy decreased 8 to 48 times. Protective effect of high-level of pegylation helps to remove PLP dependence. PEG-rMGL demonstrated a significant decrease in antigenicity [146]. The specific activity of PEG-MGL increased with DTT [147]. Although *L*-methionase from bacterial (prokaryotic) origin has immunogenic issues that can be overcome by PEGylation and by other methods such as deimmunization by combinational T-cell epitope removal using neutral drift [148].

9. MGL Cloning

MGL was used for methionine depletion *in vivo* [149]. Bacterial enzymes from various sources have been purified and tested as methionine depleting agents against cancer cell lines. The *P. putida* (pMGL) source was selected for therapeutic applications due to its high catalytic activity, low K_m , and a relatively high k_{cat} value [17]. The reaction mechanism characterized by using site-directed mutagenesis [150, 151]. The gene(s) for MGL was/were cloned into suitable host cells (Table 3).

TABLE 3: Molecular cloning and functional characterization of MGL gene in various expression systems.

Gene from	Host strain for expression	Selectable marker	References
<i>P. putida</i>	<i>E. coli</i> MV1184	Ampicillin	[35]
<i>P. putida</i>	<i>E. coli</i> BL21 (DE3)	Ampicillin	[36]
<i>P. putida</i> ICR3460	<i>E. coli</i> JM109	Tetracycline	[37]
<i>P. putida</i>	Lung cancer cell line H460	GFP fluorescence, Penicillin, Streptomycin	[38]
<i>P. putida</i>	Human lung adenocarcinoma epithelial cell line (A549 cells)	G418 (Geneticin)	[39]
<i>P. putida</i> ICR3460	<i>E. coli</i> JM109	Tetracycline	[40]
<i>P. putida</i> ICR3460	<i>E. coli</i> JM109	Tetracycline	[41]
<i>Trichomonas vaginalis</i>	<i>E. coli</i> M15pREP4	Ampicillin and Kanamycin	[42]
<i>Trichomonas vaginalis</i>	<i>E. coli</i> M15[pREP4]	Ampicillin and Kanamycin	[43]
<i>Treponema denticola</i> ATCC35405	<i>E. coli</i> BL21	Ampicillin	[19]
<i>Entamoeba histolytica</i>	<i>E. coli</i> BL21	Ampicillin	[22]
<i>Kluyveromyces lactis</i> CLIB 640	<i>E. coli</i> DH10B	Ampicillin	[44]
<i>Arabidopsis</i>	<i>E. coli</i> BL21	Carbenicillin	[24]
<i>Brevibacterium linens</i>	<i>E. coli</i> DH5 α	Ampicillin	[45]

10. Future Prospective

The unique catalytic reaction of MGL and its limited distribution in pathogens but not in human make this enzyme a promising target to design novel chemotherapeutic agents. Tumour cells show enhanced methionine dependence/requirement in comparison to the normal cells. The greater requirement of methionine by rapidly growing tumour cells supports high protein synthesis and regulation of DNA expression yet it can be exploited by the use of methionase-based therapy to rapidly deplete the cancerous cells. Thus the forced restriction of methionine may be an important strategy in cancer growth control particularly in malignant/cancers that exhibit dependence on methionine for their survival and proliferation. Currently fusion proteins (consisting of *L*-methionase linked to human annexin-V) may have an advantage in comparison to other approaches as they show application in specifically targeting tumour cells without affecting the normal cells.

Conflict of Interests

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

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