IJBPAS, December, 2020, 9(12): 3218-3247

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

THE THERAPEUTIC VALUE OF LYSINE AGAINST CANCER: A COMPREHENSIVE REVIEW

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Received 12th April 2020; Revised 3rd May 2020; Accepted 27th May 2020; Available online 1st Dec. 2020

https://doi.org/10.31032/IJBPAS/2020/9.12.5181

ABSTRACT

Current treatment methods of cancer include surgical intervention and the usage of chemosynthetic drugs or natural products. However, the synthetic drugs show broad-spectrum cytotoxicity and a higher dose for cellular inhibition. Therefore, using an amino acid like Lysine for the cancer treatment raises a new hope, as this therapeutic molecule bears no cytotoxicity towards the normal cells and works by altering the cellular metabolism from the very first stage of cancer development. Lysine was found to be significant when applied as a single moiety or in a derived form and also in a combinational form with the other drugs. This review elaborates on the current state of development, recent advancement of this amino acids highlighting the application of Lysine against several cancer cells. Overall, the effectiveness of Lysine and the molecules that it targets for the inhibition of cancer proliferation emerge a new area in the anticancer research, which proves Lysine as a potential aspirant drug in cancer therapy.

Keywords: L-lysine; cancer; cytotoxicity; apoptosis; therapy

INTRODUCTION

Cancer is the second leading cause of human death after cardiovascular diseases **[1]**. Almost 90% of cancer treated deaths are due to the metastasis of cancer cells. Cancer cells through their metastatic ability cause 90% of all cancer-related death **[2]**. Although struggling efforts against cancer have grown tremendously in the past few years, still it is the leading cause of death in economically developed countries. An increase in the worldwide death rate was found in cancer patients which are 7.1 million and 8.2 million in 2007 and 2012, respectively **[3]**. By 2030 the global burden is expected to grow to 21.7 million new cancer cases **[4]**. Several approaches, including surgery, chemotherapy,and radiation therapy are available for the treatment of cancer, but they often lack target specificity that compromises therapeutic benefits **[5]**. Development of tumor resistance against the available synthetic drugs together with the patient noncompliance invokes the requirement of safe and newer drugs and for the same, extensive research on several synthetic drug molecules is being undertaken for cancer drug discovery. Thus the imperatives for novel drugs for cancer therapies with improved specificity and safety are highly desirable.

One of the cancer treatments that seem to custom Controlled Amino Acid Treatment (CAAT). CAAT works by restricting certain amino acids from the diet to combat the growth and reproduction of the cancerous cells. However, the treatment model of this appears to be target-specific, noncytotoxic towards normal cells or tissues and also easily sustainable in normal physiological circumstances. Amongst the amino acids, lysine was found to be more appropriate over the others. It is having a simple chemical structure and good biochemical properties for the treatment of cancer cells. Having simple in structure and for some other biochemical properties lysine seems to be the most suitable amino acid for the treatment of cancer cells. This review opens up the role of lysine as an appropriate anticancer agent for the improvement of modern science in anticancer chemotherapy.

Benefits and Usage of CAAT as Cancer Therapeutic Agent

Amino acids used in CAAT studies fabricating good results as they are inoffensive to the normal cells. Although there are so many other explanations for their usage. Using amino acids as CAAT is demonstrating effective incase of studies as it is target-specific, non-cytotoxic concerning thenormal cells and tissues. However, this is not the only cause of why amino acids are used for the treatment of cancer (**Table 1)**. Amino acids have also

different effects on cells. Some help them to grow and imitate while others keep them healthy and true to the DNA blueprint entrenched in the cell at its construction. There deceptions the prominence of using CAAT against cancer cells, as they can moderate the metabolism and associated pathway of cancer cells.

Table 1: Alteration of amino acid management pathways in cancer

Amino acid metabolism			
Gene	**Function**	**Cancer alteration/relevance**	**References**
GLS	**Rate limiting step in glutaminolysis**	**Increased translational efficiency downstream of MYC, miR-23a/b**	**[98]**
PHGDH	**Rate limiting step in serine biosynthesis**	**Genomic amplification and over-expression**	**[99][100]**
SHMT2	**Diversion of serine into mitochondrial one-carbon metabolism**	**Over-expression drives hypoxia resistance**	**[99][101][102]**
GLDC	**A key component of the glycine cleavage complex**	**Prevents toxic glycine accumulation, drives broad metabolic changes**	**[102][103]**
ASNS	**Asparagine biosynthesis**	**Increased expression in glioblastoma**	**[104]**
Amino acid sensing			
mTOR	**Protein kinase controls translation in response to nutrient sufficiency signals**	**Activating point mutations, amino acid starvation fails to inactivate mTORC1**	**[105] [106]**
FLCN	**the mTORC1 positive regulator, GTPase activating protein for RAG C/D**	**Loss of function mutations in Birt–Hogg–Dubé syndrome**	**[107][108][109]**
DEPDC5	**GATOR1 component, a negative regulator of mTORC1, GTPase activating protein for RAGA/B**	**Deletion of 22q12.2, amino acid starvation fails to inactivate mTORC1**	**[110]**
NPRL2	**GATOR1 component, a negative regulator of mTORC1, GTPase activating protein for RAGA/B**	**Deletion of 3p21.3, amino acid starvation fails to inactivate mTORC1**	**[110]**

A brief overview of L-Lysine and its advantage over the other amino acid

L-lysine is an essential amino acid and we have tobe contingent on animals for its procurement in the body. It is having an amended anticancer effect than other amino acids.L-lysine is an indispensable amino acid, not produced in an animal's body, but is originate in nature still, an-ingenious plotline as it has the most advantageous over the other amino acids when being used as anticancer agents. Upon intake, L-lysine encourages healthy tissue function, growth,and healing that progresses the immune system. L-lysine is most effective in oral supplements. It is very convenient as an anticancer agent inside the body and it is under unceasing research. As a natural disease-fighting agent, L-lysine benefits the human body in a diversity of ways, many of which are only recently experiencing research.

L-Lysine Help in the Treatment of Cancer

In the contemporary drug distribution research targeted drug transfer can be

accomplished by using soft neutraceuticals like L-lysine as they do not disturb the normal cellular function. In 2007, researchers at Florida State University studied the effects of "lysine conjugates" on damaged strands of DNA, like the ones found in cancer. Essentially, this ingredient can localizethe damaged strand by recognizing "cleavage" in the situation a damaged spot and cause the respite of the strand to cleave tear a part additionally. The cell is typically incapable to repair this damage, leading to apoptosis; the suicidal death of cells.

The anti-neoplastic activity of Poly-L-lysine

Numerous studies have specified that Poly-L-lysine bears some activity against murine tumors **[6]**. Poly-L-lysine has also been found to exhibit a huge number of unique membrane belongings which comprise the aptitude to improve the cellular uptake **[7]** specifically in the agglutination of lymphocytes of the cancer patients **[8, 9]**.

These possessions are possibly accompanying with the polycationic character of Poly-L-lysine and conceivably due to precise interactions on the cell membrane. Poly-L-lysine exhibited noticeable concentration - dependent cytotoxicity to HeLa cells in culture as well as anintense antineoplastic effect against Ehrlich ascites carcinoma, Dalton's lymphoma ascites cells and Sarcoma-180 tumorcells in White Swiss mice **[10]**. The results indicate that poly-lysine is cytotoxic against cancer cells, but it has some selective possessions because at intermediate concentrations it can produce remission from Ehrlich ascites without producing much toxicity. This implies that, at the time of poly-lysine addition, protein, DNA, and RNA synthesis can be completely inhibited. The important point, however, is that treatment with poly (L-lysine) at 20 µg/ml, a minimum 75% of the cells remained viable even though their DNA, RNA, and protein synthesis had been stopped for the longer period. Moreover, it was found that poly-lysine had several other properties on HeLa cells in culture. Histologically, at low poly-lysine concentrations that did not significantly affect subsequent cell viability for the shorter time period, but its effects for the longer time period and cells viability significantly decreased, the cells became shrinkage and apoptotic bodies forms. At higher concentrations of poly-lysine, the cells were lysed and their components covered the surface of the plate as diffuse crenated discs. Under these circumstances, the cells were not viable and apoptotic bodies formed. Poly-L-lysine has profound cytotoxic effects. In addition, it is found to be most effective against Ehrlich ascites carcinoma cancer cells induced poly-lysine is very effective in preventing tumor

growth in mice. Our studies indicate that initially, the L-isomer is much more effective at preventing cell growth that is the D isomer. Probably the growth of cancer cells is inhibited by poly-L-lysine due to its ability to increase the efflux of small molecules that are required for RNA and protein synthesis. For example, furthermore to the increased efflux of potassium, it was found that poly-lysine indorses the leakage of inorganic phosphate, carbohydrates, free amino acids, small peptides, and adenosine 5'-monophosphate into the supernatant **[11]**. Previous researchers **[12]** disclose that poly-L-lysine, as well as other poly-cations such as poly-L-ornithine and poly-L-arginine, can trigger membrane phospholipase A2 in 3T3-4a Swiss mouse fibroblasts. These findings deliver a fascinating mechanism by which poly-lysine may disturb membrane permeability to small molecules by endorsing the hydrolysis of membrane phospholipid.

Based on the surveillance, the scientists proposesome model for the communication of poly-lysine with cancer cells. Those are;Poly-lysine, either poly (L-lysine) or poly (D-lysine) dilemmas forcefully to the cell membrane and induces morphological changes. Secondly, accompanying such binding is a rapid leakage of small molecules across the cell membrane, perhaps promoted by phospholipase A2 activation. Then concomitant with the poly-lysine binding is the breakage of nuclear DNA, the rapid loss of RNA producing inhibition of protein synthesis **[13]**. When poly-L-lysine is used in growing cancer cells in the culture condition, it reasons a superior degree of inhibition for short periods than that of the D isomer. After approximately 10 hr, however, the L isomer is deceptively besmirched and the abrasion modifies itself because the cells return to control growth. In contrast, with increasing time, poly-D-lysine demonstrations time-dependenton cellular growth inhibition on other-hand caused the toxicity in EAC, HeLa and Lewis lung carcinoma-induced tumor growth inthemice model. PLL has potent activity in an *in-vivo* model especially against, induced Ehrlich ascites tumors in mice but little effect upon L1210 induced tumors.

The systemic anti-angiogenic activity of Poly-L-lysine in the form of a dendrimer

Several studies propose that the Poly-L-lysine may be used for therapy of solid tumors, and alsoin a combination with their capability to carry other therapeutic or diagnostic mediators may offer competences for the proposal of theranostic systems. The antiangiogenic activity of the Poly-L-lysine dendrimer can produce biological activity and therapeutic effectiveness in the absence of any other therapeutic agent. Methotrexate (MTX)

conjugated to PEGylated PLL dendrimers have also shown to accumulate in solid Walker 256 and HT-1080 tumorsinduced rats and micemodel **[14]**. The study by Fox *et al.,* 2009 therapeutic efficacy of PEGylated PLL dendrimer-camptothecin conjugates in C26 and HT-29 tumor models **[15]**. Further work shows that G6 Poly-L-lysine dendrimer (MW 8149 Da) canaccrue and continue in solid tumor sites after systemic administration and demonstration of antiangiogenic activity in the absence of cytotoxicity in normal tissues. The occurrence of Poly-L-lysinedendrimer at lower concentrations was originated to inhibit endothelial cell migration and three-dimensional tubule formation. G6 Poly-L-lysine dendrimer was appraised herein at 25 mg/kg and 50 mg/kg after intravenous administration via tail vein injection in mice.There is previously reported that blood half-lives of <10 min, suggesting rapid accretion of the dendrimers in the vascular endothelium, a process driven by electrostatic communications **[16, 17]**. Poly-L-lysine-dendrimer accumulation at the tumor site takes place via strong electrostatic interactions between the cationic Poly-L-lysine-dendrimer surface and the negatively charged, heparin-rich ramparts of the tumor neovasculature. Antiangiogenic activity *in-vivo* succeeding systemic administration of PLL dendrimers was primarily acquired using the Matrigel plug assay. The competence of systemically administered Poly-L-lysinedendrimer to hinder microbial development in-vivo was additionallyevaluated in the dorsal skinfold window chamber model. The gradation of vascularization and significant amendment in the emerging microvascular architecture were observed by intravital microscopy **[18, 19]**. The Poly-L-lysine-dendrimer-treated group exhibited condensed numbers of microvessels subsequently treatment with Poly-L-lysinedendrimer compared to the control group. An additional indication of the Poly-L-lysine-dendrimerantian-giogeniccom motion was provided by the important reduction of the number of $CD31^+$ cells in the tumor sections of the treated groups compared to untreated control groups. The intensification in TUNEL staining of solid tumors in C57/BL6 mice furtherdesignated that the Poly-L-lysine-dendrimer encouraged apoptosis in a large tumor cell population. To regulate whether a therapeutic consequence can be initiate from thePoly-L-lysine-dendrimerantiangiogenic activity, the B16F10 murine melanoma tumor model was used. It has been recognized by others that a decrease of microvessel density in B16F10 tumors can amend the tumor cell proliferation and lead to tumor cell demise by apoptosis or necrosis **[20]**. In this study, only two intravenous administrations of the Poly-L-lysine dendrimer were able to prime

to the postponement of tumor growth **[21]**. Poly-L-lysine-dendrimer treatment led to aninterruption intumor volume expand the time of 4.7 ± 2.5 days compared to 3.7 ± 1.6 days when preserved with vehicle control. Therefore, the Poly-L-lysine dendrimer seems comparable in deferring B16F10 tumor growth to Avastin. Many molecules have been previously described to exhibit antiangiogenic activities *in-vitro*;nevertheless,most have not been effective*in-vivo*, either due to the absence of an optimum pharmacokinetic outline or due to systemic toxicity at vigorous biological doses.

Researchers also described that, the complexation of the chemotherapeutic drug doxorubicin (DOX) with the sixth-generation cationic poly-L-lysine dendrimer (DM). DOX–DM complexation was established by fluorescence polarization measurement, proton nuclear magnetic resonance spectroscopy, and molecular modeling. Heightened dispersion of DOX–DM (at 1:10 molar), paralleled to the free DOX, into prostate 3D multicellular tumor spheroids (MTS) was established by confocal laser scanning microscopy. Additionally, DOX–DM developments accomplished significantly advanced cytotoxicity in the DU145 MTS system compared to the free drug, as revealed by progression delay curves. Incubation of MTS with low DOX concentration complexed with DM led to a significant interruption in MTS evolution compared to untreated MTS or MTS treated with free DOX. DOX–DM multifaceted maintenance was also accomplished in a Call-6 lung cancer xenograft model in tumor-bearing mice. Therapeutic experimentations in B16F10 tumor-bearing mice originate to deliver the heightened therapeutic effectiveness of DOX when complexed to DM **[22]**. This study proposes that the cationic poly-L-lysine DM molecules deliberate here could, in accumulation to their systemic antiangiogenic possessions, multifaceted chemotherapeutic drugs such as DOX and advance their accretion and cytotoxicity into MTS and solid tumors*in-vivo* **[23]**. Such a tacticproposes new competencies for the strategy of conjoining antiangiogenic/ anticancer therapeutics. Intravenous administration of only two doses at 50 mg/kg Poly-L-lysine dendrimer occasioned in determine daccretion in solid B16F10 solid tumor model, lessening in vascularization, extensive apoptosis/ necrosis within the tumor tissue, and a statistically significant reduction in tumor volume, in the absence of any outstanding histological or physiological irregularity in nontumor tissues such as liver and kidneys. The dendrimer exhibited therapeutic effectiveness compared to the commercially obtainable anti-VEGF

antibody bevacizumab (Avastin) in adjourning the growth of a subcutaneous B16F10 melanoma tumor model.

Antitumor Effect of L-Lysine as Combinational Therapy

On bladder cancer cell line

The synergistic anti-cancer effect of ascorbic acid, proline, lysine, and epigallocatechin gallate (EGCG) on several cancer cell lines in tissue culture studies wasgreater than that of the individual nutrients. The synergistic anti-cancer effects of ascorbic acid, proline, and lysine on several cancer cell lines in tissue culture studies were greater than that of the individual nutrients **[24]**. The commotion of matrix metalloproteinases (MMP) on the degradation of the extracellular matrix (ECM) plays a serious role in the development of tumors and metastasis and has been originating to correlate with the aggressiveness of tumor growth and invasiveness of cancer **[25, 26]**. Afterward, researchers have recognized the perception designated by Rath and Pauling **[27, 28]** and ensued in recognizing a novel formulation of lysine, ascorbic acid and proline which has shown significant anticancer activity against a large number of cancer cell lines, blocking cancer growth, tissue invasion and MMP expression both *in-vitro* **[29, 30]** and *in-vivo* **[31, 32]**. The detailed mixture of lysine, proline and ascorbic acid is an outstanding application for preemptive and therapeutic use in the treatment of bladder cancer **[33, 34]**. However, other studies on animal models and clinical trials are essential for more exploration of the role of nutrient supplementation in the treatment of bladder cancer.

On Pancreatic Cancer Cell Line

The dose-dependent inhibitory effect of the nutrient combination of lysine, proline, arginine, ascorbic acid, and green tea extract on the MMP-9 expression of the pancreatic cancer cells was dependable with its dose-dependent inhibition of matrix invasion **[35]**. Also, this mixture of nutrients possibly improved the constancy and forte of the connective tissue, as optimization of amalgamation and construction of collagen fibrils depends on hydroxylation of proline and lysine residues in collagen fibers. Researchers already reported that the synergistic anticancer effect of prolineand lysine on several cancer cell lines in tissue culture studies was superior to that of the individual nutrients **[28]**. Furthermore, in contrast to chemotherapy which causes undiscerning cellular and ECM damage, morphological studies of pancreatic cancer cells treated with NM presented that level at the highest concentrations of the nutrient mixture (NM), the pancreatic cells were not adversely exaggerated, demonstrating that this formulation is safe for normal **[35]**.

A derivative of L-Lysine as a Natural Source

Epsilon-poly-l-lysine (ε-PL) from Streptomyces or Kitasatospora

Epsilon-poly-l-lysine (ε-PL) produced by *Streptomyces* or *Kitasatospora* is a homopolymer of L-lysine **[36]**, and it is also decomposable, water-soluble and thermally stable. It is established to non-toxic in humans **[37]**. ε-PL is anauspicious natural antimicrobial that is extensively used to preserve packaged food in convinced countries for its broad antimicrobial activity against Gram-negative and Gram-positive bacteria, yeasts, and molds **[38]**. However, the accomplishment of this approach is also reproduced in anticancer research as ε-PL is initiate to reduce the tumor size. The resection lowers the degeneration rate and is also suitable **[39, 40]**. The compounds also exhibited better antioxidant and antitumor activity **[36]**. These findings indicate that the incorporation of ε-PL could be used in the pharmaceutical industry.

L-lysine α–oxidase from Trichoderma faureoviride

L-Lysine-α -oxidase (LO,)catalyzes the adaptation of L-lysine into α -keto-€-aminocaproic acid, H_2O_2, and ammonia with the ingesting of oxygen. The enzyme was isolated for the first time in the 1980s from *Trichoderma viride* Y244-2 **[41, 42]**. The anticancer activity of LO is closely related to L-lysine plasma concentration exhaustion **[41]**. A single intravenous injection of LO (30U/kg) into BDF_1 mice in 1 h reduced the L-lysine concentration to an indiscernibly low level. This diminished L-lysine level was continued for 12 h and then improved gradually. In addition to the enzymatic dispossession of L-lysine, the cytotoxicity of LO could be attributed to H_2O_2 production. DNA breakage by H_2O_2 may be restrained as the primary mechanism of the LO cytotoxic effect on tumor cells *in-vitro* **[43]**. the-amino acid oxidizing enzymes, LO from *T. viride* or *T. Harzianum* Rifai has a comparatively narrow range of substrates **[41]**. Only L-amino acids allow a positive charge at the end of the side radical, such as L-ornithine and L-arginine, are rehabilitated by LO, but at a much leisurely rate than L-lysine. LO was revealed to be less toxic human carcinoma cell lines, with IC_{50} values ranging from 3.0×10^{-6} to 7.8×10^{-2} U/ml (3.2×10^{-8} to 8.2×10^{-4} mg/ml). Compared with other anticancer enzymes, LO is much more active *in-vitro* than L-asparaginases or novel anticancer enzymes such as arginine deiminase and onconase **[44-46]**. The most sensitive cell line was K562 ($IC_{50}=3 \times 10^{-6}$ U/ml). LO may have been a result of the different compassions to L-lysine deficiency and oxidative stress. Although the basic level of cytotoxicity is

determined by H_2O_2 production **[43]**, possibly the sensitivity to depletion of L-lysine concentration could be the main standard for the prediction of LO effectiveness in various types of cancer. The sensitivity to growth inhibition *in-vitro* was established *in-vivo* in mice bearing LS174T and HCT116 tumor xenografts. The discoveries of this *in-Vivo* experiments confirm the possible appropriateness of LO for human colon cancer treatment and specify that the advantages shown by LO may interpret into upgraded clinical benefits deprived of increased toxicity. A specific agenda is vigorous for an actual and well-tolerated LO treatment. Optimal possessions of anti-cancer drugs with an enzymatic mechanism of action can be accomplished by administering them in numerous daily doses, which guarantee sustained exposure to the drug, but this schedule leads to increase in toxicity because of a precarious unceasing decrease of the plasma L-lysine concentration. Perhaps, conservation of doses of LO delivers effective concentrations of L-lysine for the survival of normal cells whereas cancer cells are more susceptible. The poor blood supply in a tumor does not deliver continuous ingestion of indispensable amino acids critical for cycling cells with exhaustive protein synthesis, especially in G_1. This was established by the PK profile of LO **[47]** (**Table 2)**.

Table 2: Effect of Lysine in different forms (Single, combined and derivatives) on various cancer cells

Molecules	Types of cancer cells on which effects are shown	References
Single forms		
Lysine oxidase	Colorectal cancer cell	[111]
L-lysine	Leukemia cell Bladder cancer cell Pancreatic cancer cell	[112]. [113], [114], [115], [116],[117], [118]. [119].
Poly-lysine	HeLa cell Ehrlich Ascites Carcinoma cell Sarcoma-180, Dalton Ascites Lymphoma cell	[120][121][122][123].
Combined forms		
PEGylated Poly-L-lysine	Solid Walker 256 tumor cell HT-1080 tumor cell C-26 tumor cell B16F10 murine melanoma tumor	[124]. [125][126]. [127]. [128].
Poly-L-lysine dendrimer complexes Doxorubicin	Calu-6 lung cancer cell B16F10 murine melanoma tumor	[129][113]. [128]
Derivatives of L-lysine		
ε-Poly-L-lysine (ε-PL)	HepG-2 cancer cell	[130][131][132][133].
L-lysine- α -oxidase	HCT116 tumor cell LS174T tumor cell	[134][135][134][136][137][138]. [134][139][140].

Mechanism of Action of L-Lysine as an Anticancer Molecule

L-the lysine regulates tumor necrosis fact hetor alpha (TNF-α) and matrix metalloprotein-3 (MMP-3) expression

L-Lysine is intricate in multiple biological processes including inflammatory regulation. However, rare research has spokenabout the effects of Lysine on human chondrocytes. The mRNA levels of tumor necrosis factor-α (TNF-α) and matrix metalloproteinase-9 (MMP-9) reduced when normal chondrocytes treated with Lysine. Lysinedown-regulated TNF-α, MMP-3 levels, reinstated aggrecan and collagens expressions, and further increased the aggrecan/type I collagen and type II collagen/type I collagen provisions in IL-1β-stimulated chondrocytes.In addition, Lysin's treatment reduced the protein fabrications of TNF-α and MMP-3 in encouraging cells. Lysine has been originating to have beneficial effects in diverse pathological circumstances including herpes simplex virus infection, mood disturbances, anxiety, migraine, and osteoporosis **[48-51]**. Chinese traditional medicine consumptions Lysine in combination with extracts of medicinal plants to constrain acute inflammation **[52]**. Also, Lysine further decreased TNF-α mRNA expression and protein production. TNF-α is involved in systemic inflammation and related to the acute phase reaction **[53]**. TNF-α also excites the announcement of MMP-1, MMP-3, and MMP-13 **[54]**. This study revealed that the regulations among pro-inflammatory cytokines, MMPs, and TIMPs are predominantly moderate by Lysine. IL-10 modifies the countenance of anti-inflammatory cytokines, manufacture of reactive oxygen species, and a factor of pro-apoptosis of human articular chondrocytes **[55, 56]**. Since Lysine reduced the TNF-α level in stimulated chondrocytes, Lysine may not change IL-10 production. Further studies displayedthat the productions of TNF-α and MMP-3 reduced instantaneously in IL-1β-stimulated chondrocytes after Lysine's treatment. The decrease of TNF-α and MMP-3, consequently,designates that the anti-inflammatory activities of Lysineen compass the conquest of the inflammatory pathway. An exciting discovery is the modulation of Lysine in inflammatory cytokines to un-stimulated chondrocytes. Lys did not have substantial effects on IL-1β but decreased the mRNA levels of TNF-α. It is known that IL-1 interrelates with TNF-α in various physiological and pathological circum-stances. Although IL-1β is an upstream inductor to TNF-α, TNF-α can also initiate IL-1 production in chondrocytes. Also, it was demonstrated that TNF-induced structural combined impairment is mediated by IL-1 **[57]**. Martel-Pelletier *et al.,* (1998) studied the

effects of diacerein and rhein with Lysine on inflammatory cytokines in lipopolysaccharide-treated synovium and chondrocytes. These representatives produced both dose and time-dependent reduction in IL-1β protein construction in synoviocytes, and the amount of IL-1 receptors (IL-1R) on chondrocytes **[58]**. Numerous investigates of randomized measured trials of these TNF-α inhibitors exposed the conceivable risks of cancer **[58, 59]**. Lysine enhanced hypertrophic conversion of chondrocytes under IL-1β inspiration. Lysine also reductions the mRNA levels and protein constructions of TNF-α and MMP-3. Generally the imbalance meant of cytokines and growth regulatory proteins by Lysine designate that Lysine may retain anti-inflammatory that may be one of motive on how lysine triggers cell death in cancer cells.

Poly (l-lysine) s/poly (l-lysine)-DNA complexes initiate mitochondrial-mediated apoptosis pathway

Recent studies designated thatPLL induces mitochondrial apoptosis in EAC cells, *in-vivo* **[60]**. Besides this, the molecule was also found to trigger apoptosis which occurred to be facilitated through the intrinsic pathway in DAL and sarcoma-180 induced mice model **[61]**.

On the other hand, synthetic polyamines such as linear and dendritic poly(l-lysine)s, PLL, and variations thereof, have also been used for DNA compaction and transfection with capricious **[62-66]**. PLL–DNA complexes do not professionally escape from endosomes, but gene expression still occurs **[67]**. Explanations of some researchers with poly (ethylenimine) (PEI) **[68]**, and characteristic cytotoxicity of PLLs **[64, 69, 70]**. Provided the intrinsic mechanisms behind and induced apoptosis in different types of human cell lines (Jurkat T-cells, umbilical vein endothelial cells, and THE-3 hepatocyte-like cell line) by high and low molecular weight PLL (H-PLL and L-PLL) in linear form, which isnormally used in transfection protocols. The intracellular levels of endogenous polyamines (e.g., spermine and spermidine) are strongly controlled through several homeostatic mechanisms, whereas their extreme cytoplasmic accretion (e.g., resulting from insults to anionic cellular structures) transduces a death signal to mitochondria by an oxygen-independent mechanism **[71, 72]**. Incubation of Jurkat T-cells with PLL causes early events in the apoptotic cycle **[73, 74]**. PS translocation is most rapid (30 min–1h) with H-PLL at concentrations of 20 μg/ml and above, while PS acquaintance is prominent from 5 h post-incubation with L-PLL. Similar results were also obtained with THE-3 hepatocytes and HUVECs. The activity of this protease accountablefor caspase cleavage was thoughtful particularly at 24

h-post-PLL (20 μg/ml) treatment, was irrespective of PLL type and could be inhibited by Ac-DVD (a caspase-3 inhibitor). H-PLL was more effective than L-PLL in inducing caspase-3 activation. Also, caspase-3 instigation was noticeable at 24 hpost-treatment with lower concentrations of both PLL types (10 μg/ml); this was in accord with PS expression in tandem with propidium iodide staining. Researchers also obtained similar results with PLL–DNA complexes, and also in different types of human cancer cell lines. Henceforth, both PLL types induce apoptosis, but the early exposure of PS is the result of plasma membrane damage and phospholipid re-shuffling subsequent electrostatic interaction with PLL and PLL–DNA complexes; early PS expression was not affected in the presence of Ac-DVD and therefore is autonomous of caspase 3 activation. Here, it has been used PLL–DNA complexes that express positive zeta potential values (PLL: DNA of 3:1), thus impersonating previous transfection protocols. Mitochondria are whispered to be a central supervisory element in stress-induced cell death. Stress-induced inducements trigger mitochondrial permeabilization and cause the announcement of proapoptotic proteins such as Cyt*c* and Smac/DIABLO from mitochondrial intermembrane space **[75, 76]**. The results in show significant accumulation of Cyt*c* in Jurkat T-cell cytoplasm at 24 h post-PLL (both in free form as well as DNA-bound) treatment. However, more Cyt*c* was released with H-PLL treatment than L-PLL **[77, 78]**. In the case of H-PLL, the extent of Cyt*c* release was decreased by approximately 50% in the presence of a cell-permeable Bax channel blocker **[79]**, but interestingly inhibition of Bax channel-forming activity had no effect on Cyt *c* release from mitochondria by L-PLL. Similar observations were made with both free and DNA-bound PLLs. As a control experiment, Bcl-2 inhibition **[80]** accelerated cell death in all cases. Therefore, it has been concluding that H-PLL and L-PLL initiate mitochondrial-mediated apoptosis differently. In line with increased cytoplasmic Cyt*c* accumulation and concomitant with caspase-3 activation, they also detected significant caspase-9 activity thus confirming the involvement of mitochondrially mediated apoptotic pathway. Indeed, MMP in Jurkat T-cells was dramatically altered in a time-dependent manner. Nuclear accumulation of PLL–DNA complexes were previously suggested to occur within a few hours of incubation **[81, 82]**, where PLL may induce genotoxic stress. Cyt*c* release from mitochondria by L-PLL and H-PLL (L-PLL-mediated Bax-independent and partially Bax-dependent H-PLL triggered Cyt*c* release), they turned the attention

towards the direct effect of PLLs on mitochondrial membrane permeabilization and physiological functions, using freshly isolated rat liver mitochondria. H-PLL induced a progressive decline in the rate of DNP uncoupled respiration and partial mitochondrial depolarization. H-PLL may interact directly with the outer mitochondrial membrane, or at contact sites between the outer and inner mitochondrial membranes, where Bcl-2 appears to cluster **[83]**; this could modulate the concentration or distribution of cardiolipin, hence facilitating Cyt*c* release directly, altering mitochondrial energization, which enhances Bax docking **[84]**, and subsequent Bax-mediated mitochondrial destabilization/ permeabilization. Nevertheless, these studies further confirm as to why cell treatment with H-PLL induces significantly more caspase-3 activation than that of L-PLL.Intriguingly, observations of significantly higher Cyt*c* release from isolated mitochondria were restricted to H-PLL. The endogenous polyamine spermine was suggested to facilitate the release of a small fraction of Cyt*c* from isolated mitochondria through an exchangeable sub-population of this protein and without incurring mitochondrial damage **[72]**, but in light of some observations in intact cells, this mode of exit cannot explain L-PLL-induced Cyt*c* release. However, PLLs and polyarginine are known to stimulate several cellular kinases and protein phosphatases, particularly phospholipases, even at sub-optimal cytotoxic concentrations **[70]**. For instance, PLL was shown recently to induce phospholipase D activation in bovine pulmonary artery endothelial cells through the involvement of protein kinase C **[70]**. Thus, phospholipase activation, presumably through PLL-mediated plasma membrane damage and involvement of protein kinases, may lead towards the insertion of lysolipids into theouter mitochondrial membrane via recently identified lipid transfer capacity of full-length Bid **[85]**. Indeed, monolysocardiolipin is an inhibitor of mitochondrial phospholipase A2 **[86]**, thus tight binding of full-length Bid to monolysocardiolipin may lead to activation of this phospholipase too. These modifications could collectively alter the physical state of the outer mitochondrial membrane (e.g., a positive change in membrane curvature) leading to the release of a small pool of apoptogenic factors, either directly or through other mediators including PLL. Certainly, this believable mechanism may be functioning with both PLL types, but the rationality of this hypothesis remnant to be verified when mutant cells become available.H-PLL and L-PLL are not only cytotoxic through plasma membrane damage, but moreover, they are accomplished of initiating

apoptosis inversely via the mitochondrial pathway.in the absence of endosomotropic the agents, PLL and PLL–DNA complexes could induce late-phase mitochondrial-mediated apoptosis, thus substantiating their cytoplasmic attendance at around 24 h post-challenge. It would appear rational to accomplish that the induction of PLL-induced apoptosis could be contingent on a number of factors, including the cell type that is being pretentious, the intracellular levels of cysteine proteases and Bcl-2-family of proteins, and whether polyamines can afterward induce marked changes in the expression of pro- and anti-apoptotic genes since some PLL–DNA complexes are supposed to localize in the nucleus **[81]**. As poly (ethyleneglycol)-conjugated PLL **[66]** and PLL-grafted imidazoleacetic acid **[87]** can also inductee apoptosis in normal and various tumor-derived human cell lines. Understanding of polyamine/polycation-mediated apoptosis is of seriousprominence for the rational design of non-toxic delivery vectors for DNA compaction and transfection protocols, predominantly by retaining high-throughput methods **[88, 89]**.

Freebase lysine increases survival and reduces metastasis by altering the tumor microenvironment

Tumor cells are highly glycolytic uniform in the presence of oxygen and henceforth produce free protons (H^+) at a higher rate than normal cells, a spectacle known as the Warburg effect **[90]**. As importantly, the microenvironment of solid tumors is acidic and expressively affects tumor growth and invasion. Low extracellular pH principals to the improved statement of Cathepsin B and other proteolytic enzymes that consequence in the dilapidation of the extracellular matrix (ECM) **[91, 92]**. Fascinatingly tumor cells are comparatively resistant to acidic pH which is most likely due to mutations of the p53 tumor suppressor gene or additional mechanisms of the apoptotic pathway **[92]**. These explanations have led to the acid-mediated invasion hypothesis, which suggests that H^+flow movement along absorptions gradients from the tumor into the peritumoral normal tissue instigating normal cell death and ECM degradation. Cancer cells, which are acid-adapted, are then able to invade into the injure neighboring normal tissue. Acidic pH has been exposed to pre-position cancers to augmented aggressive and metastatic phenotypes in animal models. An acquaintance of tumor cells to acidic growth circumstances preceding to intravascular injection considerably upsurges their competence to metastasize **[93]**. Here scientists investigate the impending role of lysine, a freely obtainable amino acid with a pKa of 10, in buffering tumors and plummeting metastases. In part, this is due to districts of

inadequate perfusion foremost to inadequate oxygen supply (hypoxia), which necessitatesthe up-regulation of glycolysis to preserve ATP levels. Although the glycolytic phenotype is supposed to be a near-universal phenomenon in cancer cells, it has not been precisely labeled in PC3M. Therefore, It has been first examined the metabolic state of PC3M cells in assessment to a normal prostate cell line (PCS) *in-vitro*. Based on the *in-vitro* results;it has been scrutinized the prospective role of a systemic buffer, lysine, in hindering the expansion of metastases from the PC3M cell *in-vivo*. The researchers found, dependable with our estimates, that lysine considerably prevents the progress of metastases and prolongs endurance. pH plays asignificant role in virtually all steps of metastasis **[94, 95]** and metastatic disease in prostate cancer is consistently fatal. Numerous clarifications and mechanisms couldsubsidize to the consequence of buffers on tumor metastasis. Acid-mediated incursion can occur via obliteration of the extracellular matrix, which is endorsed by proteases and glycosidases. Metalloproteinases (MMP-2 and MMP-9) are supposed to be perilous for invasion and extravasation **[96, 97]**. MMPs are a family of proteolytic enzymes that reduce the extracellular matrix and junctional proteins and further upsurge endothelial permeability **[96]**. Low pH up-regulates angiogenic factors such as vascular endothelial growth factor (VEGF) and interleukineight (IL-8) motivating neo-vascularization and indorsing metastasis. Thus, it is conceivable that lysine can converse acidosis and subsequently reduce proteolytic enzyme activity and or angiogenesis, which will central to the reticence of extravasations, and colonization of circulating tumor cells reducing efficacious metastasis (**Table 3)**.

Table 3: Various mechanisms of cancer cell death by Lysine

Types of mechanisms by lysine for apoptosis	References
Regulates TNF-α and MMP-3expression	[141][142][143][144][145][146][147][145][148][149][69][150][151][152][153][154][155][156][157][158][151][152][153][154][75][77]
sMitochondrial-mediated apoptosis	[12][121][79][80][81][82][159][84][85][86][87][88][89].
Reduces metastasis by altering the tumor microenvironment	[105][106][160]

CONCLUSION

It is apparent that amino acid treatment against cancer will be in the majority in the 21st century. Thus, the discovery of the efficacy of L-lysine will certainly show its effectiveness against tumors more than the other amino acids. The biggest advantage that we have that Lysine can be cytotoxic, apoptotic against cancer cells in a proper dose-dependentmanner, which alters the metabolism of the cancer cell. On the other hand, it also shows its efficacy in several modes of application. Being single or in derived from and a combinational form,

lysine proves itself to be target-specific, cytotoxic against cancer cells sparing its normal counterparts **(Figure 1)**. However, if it could be coated through nanoparticles then it might show their effectiveness in a more defined way and lower doses. Therefore, further research on the efficacy of Lysine is to be made so that to explore a new horizon in the field of anticancer research.Thus, this review depicts several efficacies of L-Lysine, which might show a new hope of using Lysine in a broader spectrum. More clinical trials have to be made for proper evaluation of lysine in the battle over cancer then only it can provide new hope against anticancer drug development.

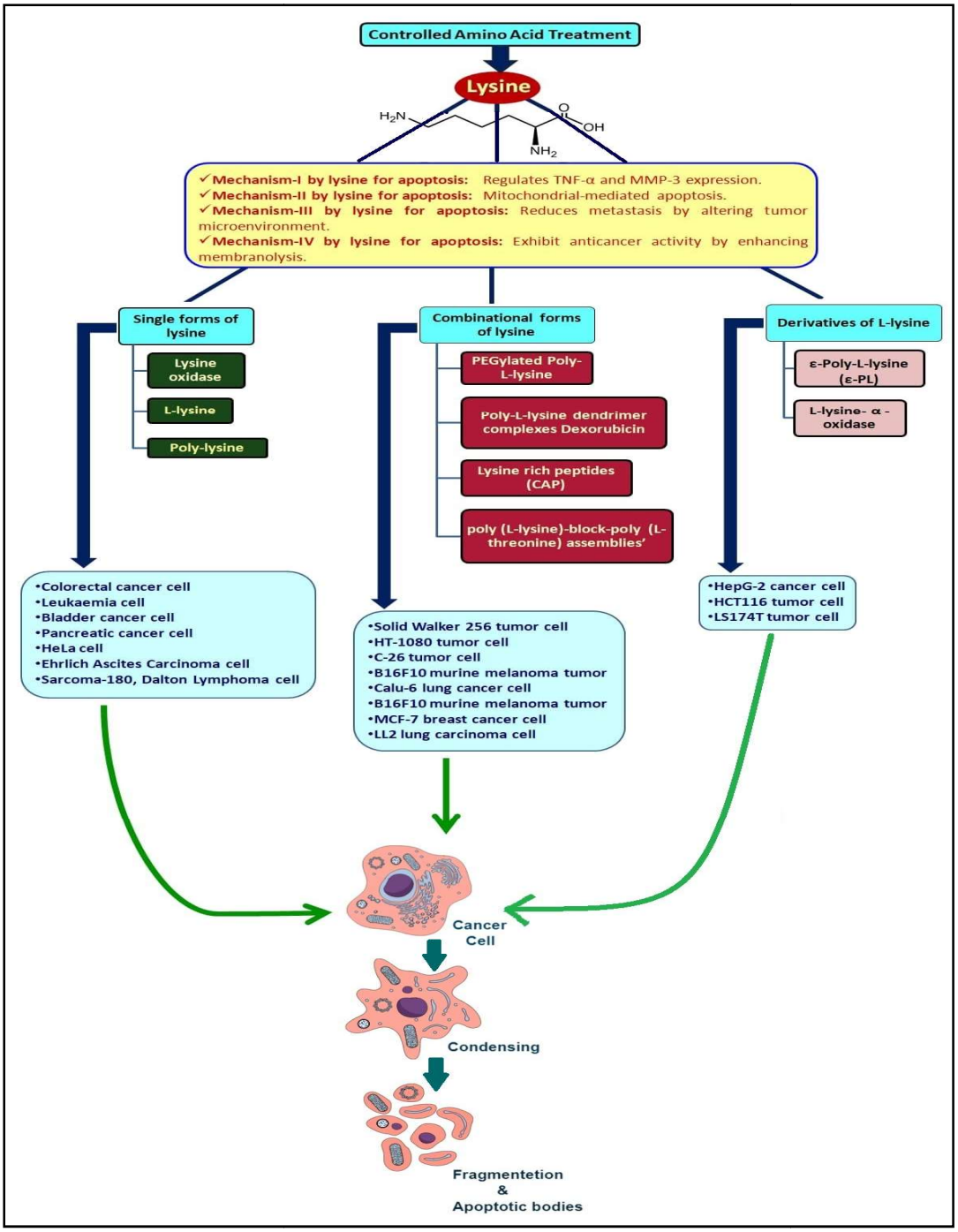

Figure 1: Effectiveness of L-lysine triggering the cancer cell death

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

University of Grant Commission, New Delhi, India, sanctioned toSouvik Debnath through UGC-BSR Research Fellowship in Science for meritorious student's fellowship scheme.

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