Research Paper

Epigenetics changes affecting L-asparaginase therapy in human leukemia: a mini review

Solmaz Moosavi [a] , Malihe Mohammadi [*b] , Milad Lagzian [c]

[a] Department of biology, Faculty of basic science, University of Sistan and Baluchestan, Zahedan, Iran
solmaz.moosavi@pgs.usb.ac.ir
[b] Department of biology, Faculty of basic science, University of Sistan and Baluchestan, Zahedan, Iran
[c] Department of biology, Faculty of basic science, University of Sistan and Baluchestan, Zahedan, Iran
m.lagzian@science.usb.ac.ir

Abstract

ASNase (L-asparaginase), as an effective anticancer component, is used in chemotherapy and treatment of ALL (acute lymphoblastic leukemia) and natural killer (NK)/T-cell lymphoma. In contrary to significant efficiency of ASNase hypersensitivity or allergy is the most common asparaginase-associated toxicities in treatment of pediatric and adult, which leads termination of ASNase therapy in ALL patients. Additionally, resistance to treatment is another obstacle in ASNase therapy, which consequently ALL relapse is occurred in result of leukemic cells resistance to treatment. A reciprocal correlation between asparagine synthetase (ASNS) expression and sensitivity to ASNase treatment is reported in ALL cells, that ASNS levels may deactivate the ASNase therapy effects. Epigenetic changes besides genomic modulation, gene expression profiling alterations and genetic polymorphism have an effective role in ASNS expression and cellular resistance to ASNase consequently. Recent studies have shown DNA hypermethylation in ASNS promoter, which named as 'silent inactivation', prevents it's transcriptional expression following asparagine depletion. So, epigenetic modifications influence chemotherapy response in ALL patients and have an impressive role in achieving new therapeutic approaches. In this review we focused on the known epigenetic changes in ASNS expression in ALL cells and also prospect to the epigenetic efficacy such as demethylating agents to combined treatment that could modulate the sensitivity and resistance to ASNase therapy in ALL patients.

Keywords: Asparaginase, acute lymphoblastic leukemia, hypersensitivity, resistance to treatment, DNA methylation**,** asparagine synthetase**.**

1. Introduction

Acute lymphoblastic leukemia (ALL) is an aggressive type of blood cancer, which characterized by a malignant transformation and proliferation of lymphoid progenitor cells in the bone marrow, blood and extramedullary sites. ALL is accounting for 30% of all malignancies in children and considered a major pediatric cancer in developed countries(1,2). Multistep pathogenesis leads leukemic transformation including at least two or numerous genetic abnormalities and the altered proteins of which drive these cells into uncontrolled cell growth and clonal expansion(3). Recently, treatment of children and also adults with ALL has improved significantly due to chemotherapeutic agents, which bacterial L-Asparaginase (ASNase) has played a prominent role among them (4,5).

ASNase as an anti-leukemia drug is the first therapeutic enzyme for line treatment of ALL with

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∗ Correspondence e-mail:
mmohammadi@science.usb.ac.ir

antineoplastic properties by preventing metastasis from solid tumors. Also there is tendency to using ASNase for non-Hodgkin lymphoma treatment, especially in combination with other chemotherapeutic agents for the treatment of natural killer (NK)/T-cell lymphoma[1,6,7] . The first discovery of the tumor-inhibitory properties of ASNase began in the early 1950s, then it has been the basis of pediatric ALL regimens since the 1960s and today, ASNase is used also to treatment of adult ALL patients [1,8].

Bacterial ASNase is a large protein and therefore its administration to ALL patients has accompanied the potential of an immune response by anti-ASNase antibodies progress. These antibodies decline the enzymatic activity by binding to the ASNase molecule and initiate a number of downstream effects including the development of clinical allergy which have been associated with IgG and IgE antibodies in ALL patients with ASNase administration[4,9]. In another hand, it has been reported that all commercial ASNase preparations are capable of eliciting an immune response by producing anti - ASNase antibodies[10]. Strongly, the reason of renewed interest in ASNase administration of adult patients is the lower rates of adverse events by recent improvements in the native *E. coli* ASNase purification and also the availability of *Erwinia* ASNase which isolated from *Erwinia chrysanthemi*, as well as the introduction of longer-acting pegylated ASNase [11,12].

Although the survival rate of patients treated with chemotherapy drugs such as ASNase is significant, but hypersensitivity is the most common treatment-related toxicity in about 15% of ALL patients[1,7]. Besides of hypersensitivity, in about 30% of patients may develop coagulation disorders and hepatotoxicity while pancreatitis occurring in up to 2% of patients, among both pediatric and adult populations. Other toxicities, such as fever, nausea, vomiting and anorexia were observed in the earlier adult trials and attributed to the presence of other bacterial proteins and impurities in the native *Escherichia coli* (*E. coli*)-derived drug [1,4,5]. Nevertheless, hypersensitivity remains a significant problem, often requiring the termination of ASNase without completing the entire course of treatment. So, In addition to clinical hypersensitivity, subclinical hypersensitivity (i.e., silent inactivation), or other treatment-related toxicity, is related to the poor results observed in the treatment of ALL patients [5].

Moreover, Although ASNase therapy leads the high survival rate of patients treated with chemotherapeutic drugs, but ALL relapse is significant about one quarter of children and more than half of adults, which is result of leukemic cells resistance to treatment[7]. Also, It is observed that ALL cells are resistant to ASNase therapy which be linked to high level of *ASNS* expression[7]. Many lymphoblastic leukemia cells are unable to synthesize sufficient concentrations of asparagine de novo, due to has little or no expression of the enzyme asparagine synthetase (*ASNS*). ASNase deprives ALL cells of asparagine and so depletes serum levels of this critical amino acid [13]. *ASNS* cellular expression high levels allow the production of asparagine and may also be linked to resistance to ASNase therapy[5]. In other words, ALL cells are sensitive to asparagine depletion and use of ASNase as the asparagine-depletion enzyme is an important treatment option in patients. Based on many studies, there is a correlation between ALL outcome and epigenetic aberrations such as DNA methylation [5,14,15]. It has been recently shown DNA hypermethylation in *ASNS* promoter prevents it's transcriptional expression following asparagine depletion that indicates the epigenetic modification has an impressive role in achieving new therapeutic approaches [14]. So, the sensitivity of leukemic cells to ASNase has been increased by down regulation of, thus routinely it is an agent used in ALL induction therapy [16]. In this review we focused to investigate a possible role of this epigenetic alteration to ASNase sensitivity of ALL cells in following of *ASNS* expression changes, and also prospect the efficacy of demethylating agents to combined treatment that could modulate the sensitivity and resistance to ASNase in ALL patients [5,17,18].

2. L-ASPARAGINASE

L-asparaginase (L-asparagine amidohydrolase, ASNase, EC 3.5.1.1) is a purified bacterial enzyme that catalyzes the hydrolysis of L-asparagine into asparatic acid and ammonia through depleting circulating asparagine [14,19,20]. The mechanism action of ASNase leads to diminish of asparagine, and so, the death of the leukemic lymphoblasts[21]. Currently, three different types of ASNase are used in clinical applications: native and pegylated form derived from *Escherichia coli* (*E. coli*-ASNase and PEG- ASNase, respectively), and an enzyme isolated from *Erwinia chrysanthemi*, known as *Erwinia* asparaginase (*Erwinia*- ASNase), antigenically distinct from *E. coli*-derived ASNase forms [10].

Asparagine levels maintain adequate by competent ASNS activity in most tissues and also asparagine depletion upregulated the enzymatic activity [22]. ASNS (asparagine synthetase) is an aminotransferase enzyme which encodes by ASNS gene and catalyzes the biosynthesis of the amino acid asparagine (Asn) from aspartic acid (Asp) [22] . Healthy cells, in contrary of tumor cells, have sufficient amounts of ASNS, and thus, are able to

synthesize asparagine de novo (11,23). Primary ALL cells and many ALL cell lines are usually sensitive to asparagine depletion and exhibit a particularl low level of ASNS expression in lymphoblasts that leads to the depletion of plasma asparagine (22). Proliferation and survival of leukemic lymphoblasts depends on protein synthesis of exogenous asparagine, while as mentioned tumor cells are unable to synthesize enough L-asparagine for their maintenance and accelerated growth because of ASNS gene absence and lack or low activity of asparagine synthetase. Hence cellular functions of leukemic lymphoblasts compromises and leads to cell death (1,11,24–26). So, Normal cells ASNS can produce L-asparagine from L-glutamine, thus are not dependent on extracellular asparagine. But leukemic cells inhibit protein synthesis due to deplete the circulating pool of L-asparagine by ASNase and catalyze this to L -aspartic acid and ammonia, which cause cell cycle arrest in the G1 phase and ultimately apoptosis induction (Thomas and Le Jeune, 2016). It is generally thought that the expression of *ASNS* is low in acute lymphoblastic leukemia (ALL) cells, rendering ALL patients sensitive to the treatment of L-asparaginase. Adaptation of L -asparaginase total dose, potentially accompanied with a reduced incidence of side-effects (Jiang et al, 2019) . Therefore, there is a reliable correlation between ASNS transcriptional expression and ASNase sensitivity in leukemic cell lines, and can be an essential component of most pediatric therapeutics protocols (22).

Most research on ASNase-resistant cells has shown an increasing of ASNS mRNA and protein expression, decreasing of asparagine efflux through Na $^+$-independent exchange system, a production of aspartic acid as a substrate for asparagine synthesis by ASNS via transamination, a higher glutamine synthetase (GLNS) activity through posttranscriptional regulation, an activation of the glutamine transporter. In addition, inhibition of ASNS or GLNS expression or activity can sensitize resistant cells to ASNase (7).

As well as, most studies demonstrate that at the transcriptional level there is relevance between ASNS expression and ASNase sensitivities. Although transcriptional expression might not be the best marker of ASNS activity because ASNS mRNA and intracellular protein concentrations relevance has not been established (22). However, *ASNS* cellular high expression levels which allows the production of asparagine in the leukemic cells, lymphoblasts, and mesenchymal cell lines of patients may be neutralized the ASNase effects and lead to treatment resistance (27). ASNS low expressing cells can induced transcriptional of ASNS and therefore adapted to ASNase treatment (14,19). T-lineage ALL and B-lineage ALL has a low

expression of cellular ASNS, that explaining the higher sensitivity to ASNase treatment in B-lineage ALL (5). In this regard some investigations have suggested that the cause of low expression of ASNS in certain subgroups of ALL is due to the hypermethylation of the ASNS promoter that explaining the high sensitivity to ASNase treatment (5,28–30). Although, ASNase preparations may cause rapid inactivation of the ASNase resulting in suboptimal asparagine depletion and do not always leads to clinical hypersensitivity. This is frequently named as 'silent hypersensitivity' or 'silent inactivation' and may arise in about 30% of the patients and it is characterized by circulating antibodies without clinical appearance (10).

3. EPIGENETICS

Epigenetics is a biological system that involved in phenotypes of organism and occurs due to differential regulation of genes and modification of gene expression without alterations in the DNA sequence (despite to genetic mutations) (31). The epigenome includes DNA methylation, histone modifications, and RNA-mediated processes, and any disruption to these processes can lead to pathogenic conditions (32). Consequently, improve therapeutic response that limit cancer progression and also finding novel targeted therapies is depend on a broader understanding inclusive of epigenomics (31). Epigenetic changes are fundamental agent in B- and T-ALL lineage in both adults and children (32).

In the last years some studies highlighted that epigenetic aberrations may be determinant in inducing resistance to chemotherapy and poor outcome in T -cell acute lymphoblastic leukemia (T-ALL) (28)**.** Further, recently, the promising attempts are processing the utilization of demethylating agents; therefore provide new treatment options in T-cell malignant disorders (5). It was investigated that epigenetic changes besides genomic modulation and alterations gene expression profiling of *ASNS*, and genetic polymorphism has role in cellular resistance to ASNase (17).

3.1. DNA Methylation Changes in ALL

DNA methylation is the most studied epigenetic modification in ALL, involving the covalent addition of a single methyl group to cytosine residues, primarily within a CpG dinucleotide context (4,16,32). Mammals promoter regions is available about 70% in CpG islands (32,33). ALL cells can cause resistance to asparagine depletion by inducing the *ASNS* expression through DNA methylation at the promoter region (14). DNA methylation status at the promoter regions commonly correlates with gene expression silencing (32,34) . One of the epigenetic

mechanisms for genes silencing is the methylation of CpG islands of genes, that had been observed an aberrant methylation of CpG islands in a cohort of genes in tumor cells (28). Hypermethylation of the *ASNS* promoter is the one mechanism implicated in gene silencing of *ASNS* that is a common characteristic alteration of ALL and other types of leukemia in adults and children (10,32,35,36). *ASNS* transcriptional expression is inhibited by DNA hypermethylation at the *ASNS* promoter following asparagine depletion, hence leads to asparagine deficiency, which facilitates apoptosis by an ATF4-independent induction of C/EBP homologous protein (CHOP). ATF4 through a GCN2-dependent adaptive amino acid response is induced ASNS transcription when *ASNS* promoter is hypo- methylated. However, lack of ATF4 ability to recruited to the cis-regulatory element for transactivation leads ASNS promoter to hyper-methylation. CHOP induction is occurred only in ALL cells that are unable to turn on the expression of ASNS following asparagine depletion to trigger apoptosis. Although its induction correlated with intracellular asparagine deficiency and did not require the presence of ATF4 (14).

Therefore ASNS has determining the rate limiting step of de novo asparagine biosynthesis. Also, a role of *de novo* asparagine biosynthesis in mitigating the nutrient stress caused by depletion of exogenous asparagine is suggesting a reciprocal correlation between ASNS expression and sensitivity to ASNase treatment In ALL cells (14,37–39). Low expression of ASNS due to hypermethylation is mediated higher sensitivity to ASNase therapy and possibly higher frequency of ASNase-related complications (27). In other word, lower *ASNS* expression increases the high sensitivity to ASNase in leukemic cells, which is an agent routinely used in ALL induction therapy (10,16). As well as in another study, low expression of *ASNS* gene is associated with the mechanism of gene silencing in T- ALL cell lines that influenced hypermethylation of the ASNS promoter (28). The CpG island of the *ASNS* gene is methylated in both B-lineage ALL and T -ALL. A CpG island of the *ASNS* gene has been reported to be methylated in a murine lymphoma cell line, as well as the human leukemia cell lines, and these cells do not express *ASNS*; as a consequence, these cells are ASNase sensitive. Demethylating agents looks promising new treatment options in T-cell malignant disorders (13,18,40). Gene silencing mechanism in T-ALL cell lines demonstrates low expression of ASNS gene, which appeared to be associated with hypermethylation of the ASNS promoter (5,28).

3.2. Histon Modification in ALL

Histones are alkaline proteins involved in DNA organization inside the eukaryotic cell nucleus. H1/H5, H2A, H2B, H3 and H4 are the common type of histones. Histones post-translational modifications determinants the chromatin state and influences gene transcriptional states. Gene repression or activation is due to covalent histone modifications are known, such as acetylation, methylation and phosphorylation (4,32,36). Studies of the one type of leukemia cell line found that the lysine residues at position 9 (H3K9) and position 4 (H3K4) of histone H3 of *ASNS* in the CpG island were deacetylated and demethylated, respectively. These findings showed that suppression of expression of *ASNS* occurs by multiple steps. Interestingly, however, sensitivity to ASNase was thought not to be associated with the expression levels of *ASNS* in ALL cells having the t (12; 21) chromosomal alteration. In another study found that although these ALL cells expressed *ASNS* mRNA, they were significantly more sensitive to ASNase than ALL cells not having the translocation. On the other hand, the same investigators found that levels of expression of *ASNS* mRNA in TEL-AML1-negative ALL cells (t (12;21) translocation) were correlated with their ASNase resistance. Krejci et al. also showed higher expression of ASNS mRNA in TEL-AML1-positive ALL cells after ASNase treatment. The relationship between methylation of the ASNS promoter and chromosomal translocation remains to be determined (28,41–43) . To provide additional support for the epigenetic silencing of ASNS, active histone marks in several T -ALL cell lines and primary blasts were analyzed. Altogether, these results suggest epigenetic silencing of ASNS in TLX1^{+} cases by both DNA methylation and a decrease of active histones marks (22).

As mentioned before, ASNS transcriptional expression is inhibited by DNA hypermethylation at the *ASNS* promoter following asparagine depletion, hence leads to asparagine deficiency, which facilitates apoptosis by an ATF4-independent induction of C/EBP homologous protein (CHOP). ATF4 activity at the *ASNS* promoter is critically dependent to chromatin accessibility, which can switch ALL cells from an ATF4-dependent adaptive response to ATF4-independent apoptosis during asparagine depletion and may also help explain why ALL cells are most sensitive to ASNase treatment when compared to other cancers (14,44). Despite the fact that ATF4 is required for the induction of ASNS in ALL cells following asparagine depletion, activation of ATF4 through amino acid response pathway was not sufficient to drive transcription of ASNS. Indeed, DNA hypomethylation in the CpG Island at the promoter region of ASNS is a

prerequisite for ATF4 recruitment and trans-activation (14,22). In contrast, DNA hypermethylation at ASNS promoter restricts the accessibility of ATF4 to chromatin, leading to the failure in induction of ASNS and inability to synthesize asparagine de novo when exogenous asparagine is deprived. Furthermore, lack of intracellular asparagine leads to CHOP accumulation and CHOP-dependent apoptosis in an ATF4-independent manner. The transcriptional regulation of *ASNS* in tumor cells that inherit distinct chromatin modification status at a specific gene promoter it is affect to command cell fate decision. These findings will help to facilitate treatment strategies involving ASNAse in ALL patients and may accelerate development of therapeutics to better target asparagine biosynthesis in cancer (14).

3.3. Changes in miRNAs

MicroRNAs (miRNAs) are single-stranded noncoding RNAs and constitute another type of epigenetic regulation which regulate the activity of protein coding genes (32,45). Alterations of miRNA expression have been observed in children and adults B- and T-ALL (32). Different genetic subtypes of ALL and drug resistant cases have unique miRNA expression profiles (17). BCP -ALL cells standard ASNase therapy and another component accompanied with in vitro sensitivity has been studied for respective changes in miRNA profiles (45) . However, only one microRNA was related to L-asparaginase resistance, miR-454 that expressed at a 1.9-fold lower level in l-ASP-resistant cases (17,46).

4. CONCLUSION

ASNse has been shown to be the most important developmental agent in the treatment of ALL in chemotherapy. Despite the significant success rate, the main complication related to this effective treatment strategy is hypersensitivity, and ALL relapse is occured about one quarter of children and more than half of adults, which is result of leukemic cells resistance to ASNase therapy. Many efforts are being made to minimize the incidence of hypersensitivity reactions in ALL patients, and besides genomic modulation and alterations, epigenetic changes are also investigated in resistance to ASNase. One mechanism which directly modulates asparagine synthesis of ASNase resistance could be associated with *ASNS* expression. Many studies attempted to address the potential role of *ASNS* expression in ASNase therapy resistance by investigate the expression levels of ASNS enzyme before and after ASNase treatment. As a result, the ability to inducing of the *ASNS* expression is critical to reduce resistance to asparagine depletion. Thus, *ASNS* upregulation

makes ALL cells refractory to ASNase. Gene silencing mechanism in T-ALL cell lines demonstrates low expression of *ASNS* gene, which appeared to be associated with hypermethylation of the ASNS promoter. Low expression of ASNS due to hypermethylation is mediated higher sensitivity to ASNase therapy and possibly higher frequency of ASNase-related complications. Also, epigenetic silencing of ASNS is caused in TLX1^{+} cases by both DNA methylation and a decrease of active histones marks. Epigenetic is a relatively new field in molecular biology and many aspects of it, and especially its association with disease, are not well understood. However, it is clear that epigenetic changes study can help to access to efficient treatment for all patients by provide establish the appropriate strategies and can modulate the sensitivity to ASNse treatment in ALL patients.

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