Research Article

Assessment of glycine decarboxylase levels in p53-mutated B-cell lymphoma

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ABSTRACT

Objective: P53 gene mutation is a critical factor that affects many tumors including B-cell lymphoma. Regardless, it remains unclear how p53 mutations cause tumorigenesis. This study aimed to assess the relationship between glycine decarboxylase (GLDC) and p53-mutated B-cell lymphoma.

Methods: Point mutations in human lymphoma cells were detected via the PCR-SSCP method. In addition, GLDC amounts in SU-DHL-9 and SU-DHL-1 cells were evaluated by quantitative real-time PCR (qRT-PCR) and immunoblotting at the gene and protein levels, respectively. GLDC silencing was performed via siRNA technology in SU-DHL-1 cells and confirmed by western blot. In established mouse models of SU-DHL-1 and SU-DHL-9 B-cell lymphoma, GLDC gene (qRT-PCR) and protein (immunoblotting) expression levels were assessed.

Results: We found that SU-DHL-9 had no point mutations, and SU-DHL-1 and SU-DHL-8 cells revealed one-point mutation each. SU-DHL-1 cells exhibited increased GLDC mRNA and protein expression levels, while SU-DHL-9 exhibited markedly reduced amounts of GLDC protein in the siRNA group, compared with the blank- and negative-control groups. Animal experiments revealed higher GLDC gene and protein expression levels in SU-DHL-1-induced mouse lymphoma.


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Introduction

Cancer occurrence is a multi-step process of gradual change that requires a variety of oncogenes and tumor suppressor genes. In a variety of tumors, p53 mutations have been found to be correlated to carcinogenesis; and p53 gene mutations have been associated with many human cancers, with almost 50% of all cancer types expressing a mutant form of p53 [1, 2]. Regardless, it remains unclear how P53 mutation causes cancer. Lymphoma is a common malignant tumor, especially non-Hodgkin’s lymphoma (NHL). Its treatment is very difficult, and the patient might easily die. Non-Hodgkin's lymphoma is mainly B-cell lymphoma, which is mostly caused by mutations in p53 [3, 4]. In B-cell lymphoma, impressive progress has been achieved [5-7]. Present medical therapies initially control lymphoma growth. However, relapse rates are high. Previous studies have demonstrated that glycine decarboxylase (GLDC) is linked to the carcinogenesis of non-small cell lung cancer (NSCLC), providing targets for the development of new anticancer drugs [8, 9].

P53 gene has a high incidence in B cell lymphoma. Regardless, it
remains unclear how P53 mutation causes cancer. Previous studies have demonstrated that glycin decarboxylase (GLDC) is linked to the carcinogenesis of non-small cell lung cancer (NSCLC). GLDC regulates many metabolites, in glycolysis and the glycine/serine pathway, leading to specific changes in pyrimidine synthesis. Pyrimidine derivatives like thymidine, in turn, are required for nucleotide synthesis in cell proliferation [8]. GLDC induces dramatic changes in glycolysis and glycine/serine metabolism, leading to changes in pyrimidine metabolism to regulate cancer cell proliferation. Maybe glycin decarboxylase mechanisms, leading to P53 gene mutated lymphoma tumor development. To date, few studies have assessed p53 mutations for their effects on GLDC expression in B-cell lymphoma. Therefore, this study aimed to evaluate GLDC levels and p53 mutations in B-cell lymphoma.

Materials and Methods

Three human malignant lymphoma cell lines were provided by the Chinese Academy of Sciences, Shanghai Cell Institute, including SU-DHL-1, SU-DHL-8 and SU-DHL-9. These cell lines were derived from human pleural or peritoneal exudates and were all diffuse large B-cell lymphoma. These human malignant lymphoma cell lines were cultured for 3-4 days in RPMI-1640 medium, supplemented 10% fetal bovine in a humid environment with 5% CO₂ at 37°C.

I PCR-SSCP

DNA was extracted from the three above human malignant lymphoma cell lines. The high mutation regions of exon 5, exon 6 and exon 7 were amplified, and three pairs of primers were synthesized with the following sequences: exon 5, forward 5'-TACTCCTCTGCCCTCCAACAAGA-3' and reverse 5'-GTCTATCTGAGGAGCGCTCATG-3'; exon 6, forward 5'-GATTGCTCTCAGGTCTGGCCCCT-3' and reverse 5'-CAGACCTCAGGCGCTGACATG-3'; exon 7, forward CTAGGTGGCCTGCTGACTGTACCA-3' and reverse 5'-TGAGGTCTCCAGTGGTG-3'. PCR-SSCP was carried out according to a previous study[10].

Since SU-DHL-9 cells have no point mutation in exon 5, exon 6 and exon 7, this cell line was used as the control group. SU-DHL-1 cells have no point mutations in exon 5 but exon 6 and exon 7 but have one-point mutation in exon 6. SU-DHL-8 cells have no point mutations in exon 6 and exon 7 but have one-point mutation in exon 5. SU-DHL-1 cells were selected as the experimental group.

II Real-Time PCR

Total RNA was extracted from 40-80 mg of SU-DHL-9 and SU-DHL-1 cells using TRIzol reagent (Invitrogen, USA), and 1 μg of total RNA was submitted to the reverse transcription using M-MLV Reverse Transcriptase (Promega, USA). The SYBR Green real-time PCR kit (TaKaRa, Japan) was used to assess GLDC mRNA amounts on a Light Cycler 2.0 qRT-PCR instrument (Roche Diagnostics, Germany). The following primers were used: GLDC, forward 5'-CCCGGACUGUGUCGACGTT-3' and reverse 5'-CACCAACTGGGAGCAGATGGGAAAA-3'. PCR was performed for 10 seconds at 95°C and one minute at 58°C (35 cycles); and the melting curves were obtained. Data were analyzed using the 2^ΔCT method.

III Immunoblotting

Total protein extraction from SU-DHL-9 and SU-DHL-1 cells was carried out. Equal amounts of protein (40μg) were resolved by 10% PAGE-SDS and transferred onto PVDF membranes. After blocking, these membranes were incubated with GLDC polyclonal antibodies (Santa Cruz); and enhanced chemiluminescence reagents were used for detection. Then, band intensities were obtained from the developed films, and semi-quantitatively was performed by analyzing the GLDC/β-actin ratios.

IV GLDC siRNA transfection of SU-DHL-1 cells

The GLDC-specific siRNA was 5'-CCGGGACUGUGUCGACGTT-3', and the scramble control was 5'-CUGUGGCAAGCUCUGACUTT-3'Ambion Inc. (Foster City, CA, USA) provided the online design software, and GLDC-specific interference fragment siRNAs were produced by Shanghai GenePharma Co., Ltd. (Shanghai, China). Single cell suspensions were prepared from SU-DHL-1 cells in the logarithmic growth phase, and 3.0 × 10^5 cells were seeded in 12-well cell culture plates in 1.0 ml of cell culture medium. These cells were allowed to attach for 12 hours before transfection with GLDC siRNA or scramble control siRNA using Lipofectamine® 2000 (Invitrogen), according to manufacturer's protocol. Three experimental groups were designed: blank control (non-transfected SU-DHL-1 cells), GLDC siRNA interference (SU-DHL-1 cells transfected with GLDC-specific interference siRNA fragments), and negative control (SU-DHL-1 cells transfected with negative control siRNA). Cells were collected after 24 hours of incubation. Western blot was used for protein assessment in various groups.

V Animal experiments

This study was carried out based on international guidelines and was approved by the Animal Ethics Committee of Shandong University Affiliated Qianfoshan Hospital. Two- to three-day SU-DHL-1 cell cultures were subcutaneously administered (0.2 ml of medium) to 30 BALB/c nude mice (3-5 weeks; Chinese Academy of Sciences, Shanghai Lab Animal Research Center) to establish the model of B-cell lymphoma. Then, 30 other BALB/c nude mice received SU-DHL-9 cells as controls. Thirty days later, tumors manifested in the 28 mice in each group. At the end of the experiment, animals were sacrificed by decapitation, and the obtained lymphoma tissue specimens were assessed by PCR and immunoblotting, as described above.

VI Human B-cell lymphoma analyses

A total of 70 fresh human lymphoma tissue specimens were collected and stored at -80°C. Immunohistochemistry results revealed 58 cases with B-cell lymphoma. Anti-p53 monoclonal antibodies (BD Biosciences, 1/50) and the streptavidin-peroxidase (SP) technique were employed for immunohistochemistry. According to the immunohistochemical staining data, 42 fresh human lymphomas constituted the p53 positive group, while 16 cases formed the p53 negative group. GLDC protein levels were detected in the p53 positive
glycine decarboxylase expression in p53 mutated B cell lymphoma

and negative groups by immunoblotting, as described above.

VII Statistical Analysis

| Table 1: P53 gene mutation patterns in the three human malignant lymphoma cell lines assessed |
|-----------------|-----------------|-----------------|-----------------|
| Cell lines      | Exon 5          | Exon 6          | Exon 7          |
| SU-DHL-1        | -               | +               | -               |
| SU-DHL-8        | +               | -               | -               |
| SU-DHL-9        | -               | -               | -               |

Results

PCR-SSCP data revealed that human malignant lymphoma cell line SU-DHL-9 had no point mutations in exon 5, exon 6 and exon 7. Furthermore, SU-DHL-1 revealed no point mutations in exon 5 and exon 7, but one-point mutation was found in exon 6. SU-DHL-8 had no point mutations in exon 6 and exon 7, and one-point mutation was found in exon 5. The p53 gene mutation statuses of these three human malignant lymphoma cell lines are shown in (Table 1). In human malignant lymphoma SU-DHL-1 cells in the experimental group, GLDC gene expression levels were significantly higher, compared with SU-DHL-9 cells in the control group (13.48 ± 1.36 vs. 3.24 ± 0.38, \( P<0.05 \)). GLDC protein amounts in the experimental group were significantly higher, compared with those in the control group (22.63 ± 2.32 vs. 6.48 ± 0.65, \( P<0.01 \)). These findings are presented in (Figures 1). Western blot revealed that human malignant lymphoma SU-DHL-9 cells transfected with siRNA targeting GLDC had significantly decreased protein amounts, compared with that in the blank- and negative-control groups (all \( P<0.05 \), Figure 2). GLDC protein levels were similar between the negative- and blank-control groups (\( P>0.05 \)).

Animal experiments revealed that mice had significantly increased GLDC mRNA expression levels in the experimental group, compared with those in the control group (21.36 ± 2.62 vs. 6.65 ± 0.83, \( P<0.05 \)). GLDC protein amounts also significantly increased in the experimental group, compared with the control group (26.35 ± 2.63 vs. 8.42 ± 1.25, \( P<0.05 \); Figure 3). Human B-cell lymphoma GLDC protein expression levels (21.43 ± 2.34) were significantly higher in the p53 positive group, compared with that obtained in the p53 negative group (7.86 ± 0.86) (\( P<0.01 \), Figure 4).

Figure 1: Glycine decarboxylase (GLDC) protein expression in lymphoma cell lines cells.

Experiment group was human malignant lymphoma cell lines SU-DHL-1 cells. Control group was human malignant lymphoma cell lines SU-DHL-9 cells.

Figure 2: GLDC siRNA Transfected human malignant lymphoma cell lines SU-DHL-1 cells.

GLDC siRNA interference group; 2: Negative control group; 3. Blank control group.

Figure 3: Glycine decarboxylase (GLDC) protein expression in lymphoma cell lines mice lymphoma tissue.

Experiment group was human malignant lymphoma cell lines SU-DHL-1 mice lymphoma group. Control group was human malignant lymphoma cell lines SU-DHL-9 mice lymphoma group.

Figure 4: Glycine decarboxylase (GLDC) protein Expression in human lymphoma tissue.

1: P53 protein negative group.

2: P53 protein positive group.

Discussion

Despite impressive progress in cancer research [11-13] and lymphoma studies [14-17], clinicians still encounter multiple hurdles in treating malignancies including B-cell lymphoma. Multiple human tumors are associated to p53 gene mutation.
remains unclear how P53 mutation causes cancer. A lot of evidence has proved certain changes in GLDC in cancer [8]. However, few studies on the effects of p53 mutations on GLDC expression in B-cell lymphomas. The association of B-cell lymphoma and GLDC was evaluated in this study. As shown above, GLDC expression levels increased in SU-DHL-1 cells both at the gene and protein levels, compared with SU-DHL-9 cells. SU-DHL-1 cells have one-point mutation in exon 6, while SU-DHL-9 cells have no point mutations. This indicates that the p53 gene-mutated lymphoma cells increased GLDC levels. Western blot revealed that SU-DHL-9 cell knockdown for GLDC revealed significantly reduced GLDC protein levels, compared with the blank- and negative-control groups. In animal experiments, both GLDC gene and protein expression levels were higher after subcutaneous administration of SU-DHL-1 cells in mice, compared with the SU-DHL-9 lymphoma group. SU-DHL-1 cells yielded p53 gene-mutated lymphomas, while SU-DHL-9 lymphomas had no p53 mutations. Human B-cell lymphoma experiments revealed that GLDC protein expression levels were higher in the p53 positive group than in the p53 negative group. Mutated p53 have a longer survival time [18]. Therefore, Immunohistochemistry staining positive cells is usually p53 mutated cells [19]. High amounts of GLDC and other glycine/serine enzymes induce tumorigenesis. GLDC affecting carbon flow in both the photosynthetic and photosynthetic pathways [20]. Previous findings indicate that increased metabolism in the glycine/serine pathway due to GLDC or other glycine/serine enzymes can exert a potent tumorigenic effect [8].

In an unusual decarboxylation mechanism, the resulting aminomethyl moiety is instead transferred to an accessory H-protein [21]. In humans, GLDC is part of a multienzyme complex that couples the decarboxylation of glycine to the biosynthesis of serine [21]. Our previous study found that glycine decarboxylase Expression Increased in p53-Mutated B Cell Lymphoma Mice [18]. One study found that GLDC are negative prognostic factors in primary early-stage non-small cell lung cancer [22]. The other study showed that the combination of GLDC expression is an independent prognostic factor in early-stage NSCC. Our results will assist future development of therapeutic approaches targeting GLDC or exploiting tumor hypoxia [22]. The above results revealed that p53 mutations upregulate GLDC in B-cell lymphomas. These findings indicate a direct relationship between GLDC expression and p53 gene-mutated B-cell lymphoma, with inductive effects on tumor pathogenesis. Providing targets for new drugs.

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