Glycine Decarboxylase Expression Increased in p53-Mutated B Cell Lymphoma Mice

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Introduction

p53 gene mutations are associated with human tumors, and are among the most common genetic abnormalities. p53 gene mutations are often associated with aggressive tumor behavior and poor patient prognosis [1]. Regardless, the contribution of this p53 property to tumor suppression is not clear.

B cell lymphoma originates from B lymphocytes; the most common type is diffuse large B cell lymphoma which is also one of the most aggressive types [2]. p53 mutations are common in B cell lymphoma [3].

Many advances have been made in B cell lymphoma [4–6]. Current treatment can control lymphoma growth initially, but most patients relapse easily. Recent research found that the metabolic enzyme glycine decarboxylase (GLDC) is critical for tumor-initiating cells in non-small cell lung cancer (NSCLC). The link between glycine metabolism and tumorigenesis has provided novel targets for advancing anticancer therapy [7, 8]. GLDC acts in the glycine cleavage system by decarboxylating glycine and transferring a one-carbon unit into the folate-mediated one-carbon metabolism. GLDC disruption also results in abnormal tissue folate profiles; formate treatment normalizes the folate profile, restores embryonic growth, and prevents neural tube defects (NTDs) suggesting that GLDC deficiency causes NTDs through limiting the supply of one-carbon units from the mitochondrial folate metabolism [9].

GLDC appears to be an important feedback signaler that contributes to the control of the Calvin-Benson cycle and hence the carbon flow through both photosynthesis and photorespiration [10].

Whether p53 mutations influence GLDC expression in B cell lymphoma, remains elusive, and only few relevant studies have been reported. To understand the relationship between p53 mutations and GLDC expression in B cell lymphoma, we established B cell lymphoma animal models to study the above question.
**Materials and Methods**

**Animal Models**
All experiments were conducted in accordance with international guidelines and approved by the Shandong University Affiliated Qianfoshan Hospital animal ethics committee. Human Raji B lymphoma cells (Chinese Academy of Sciences Shanghai Cell Institute) were cultivated for 2–3 days in RPMI-1640 medium (Sigma-Aldrich Co., St. Louis, MO, USA), which contains 10% fetal bovine serum, 100 U/ml penicillin, and 100 U/ml streptomycin, at 37 °C, 5% CO2, and 95% moisture, and subcultured once. The density of logarithmic-phase Raji cells was adjusted to 1×10^5 cells/ml with serum-free RPMI-1640. 50 BALB/c nude mice (4–6 weeks old; Chinese Academy of Sciences Shanghai Experiment Animal Central) were subcutaneously inoculated with 0.2 ml of the cell mixture to establish a B cell lymphoma animal model. 30 days later, 48 mice had generated tumors. At the end of the experiment, the BALB/c nude mouse cells were decapitated, and the extracted lymphoma tissues were divided into 3 parts: 1 part was used to generate paraffin-embbeded specimens, 1 part was used for real-time polymerase chain reaction (PCR), and the remaining part was used for Western blotting.

**Immunohistochemical Staining**
BALB/c nude mouse lymphoma tissue was fixed in formalin solution, embedded in paraffin, and cut into sections (4–5 μm). p53 monoclonal antibody (BD Biosciences, Franklin Lakes, NJ, USA) was diluted at 1:50, and the SP (streptavidin-peroxidase) method was used for immunohistochemical staining. p53 protein-positivity was indicated by diffuse or scattered brownish yellow granules in the nucleus.

According to the results of immunohistochemical staining, the BALB/c nude mouse cells were divided into a p53 protein-positive group and a p53 protein-negative group.

**Real-Time PCR**
Lymphoma tissue derived from the p53 protein-positive and p53 protein-negative mice was grinded in liquid nitrogen. Total RNA was extracted from 50–100 mg tissue using TRizol® (Invitrogen, Carlsbad, CA, USA). RNA was briefly exposed to RNAase-free DNAase I, and 1 μg RNA was reverse transcribed into cDNA with M-MLV Reverse Transcriptase (Promega, Madison, WI, USA).

GLDC gene mRNA expression was detected using the LightCycler® 2.0 real-time quantitative PCR instrument (Roche Diagnostics, Mannheim, Germany). All PCR reagents were from the SYBR® Green real-time PCR kit (TaKaRa Bio, Shiga, Japan). The cDNA was amplified using the following primer sequences: forward: 5' GCCACCGGCACCGCCACCGC 3'; reverse: 5' GAGC-GAGCGTCGCTTTAACG 3'. β-actin was used as an internal control gene with forward sequence: 5' GTCTTTAATGTATGCTACGTC 3'; reverse: 5' TGACAG-GTGGAAAGGTCTGCTC 3'. PCR cycling parameters were as follows: 10 s at 95 °C and 1 min at 60 °C for 40 cycles followed by a thermal denaturation protocol. The mRNA expression of GLDC compared with β-actin was determined using the 2^(-ΔΔCt) method.

**Western Blot**
Total protein was extracted from the p53 protein-positive and p53 protein-negative mice, and the protein content was calculated. 50 μg total protein was subjected to electrophoresis in 10% polyacrylamide gel. Proteins were visualized by chemiluminescence reagents and GLDC polyclonal antibody (Santa Cruz Biotechnology, Dallas, TX, USA), the films were photographed, and the computer images were analyzed according to band density. Data were semiquantitatively analyzed with GLDC/β-actin (Santa Cruz).

GLDC siRNA Transfected Human Raji B Lymphoma Cells
Human Raji B lymphoma cells (Chinese Academy of Sciences Shanghai Cell Institute) were cultivated in RPMI-1640 medium at 37 °C, 5% CO2, and 95% moisture.

The sequence of the GLDC-specific interference fragment siRNA was 5' U U C U C G G A A C G U G U C A C G U T T 3', and that of the negative control siRNA was 5' U U C U C G G A A C G U G U C A C G U T T 3'. The online design software was provided by Ambion Inc. (Foster City, CA, USA) and produced by Shanghai GenePharma Co., Ltd. (Shanghai, China).

Logarithmic-phase Raji cells were routinely cultivated in a 6-hole cell culture plate (3×10^5 cells/hole) for 24 h to achieve cell monolayer growth of 30–50%. GLDC siRNA or negative control siRNA and Lipofectamine® 2000 (Invitrogen) were mixed and then added to the cells according to the manufacturer’s instructions. Phosphate-buffered saline (PBS)-treated human Raji B lymphoma cells were used as blank controls. After transfection for 4–6 h, the culture fluid was discarded and RPMI-1640 containing 10% calf serum was added to continue cultivation.

The 3 experimental cell groups were transfected over 24 h. Then, the total cell protein was extracted; the bicinechonic acid assay (BCA) was used to detect the total protein, and the protein concentration was determined. Samples were mixed with 2x SDS buffer to equal volumes and processed in a boiling water bath for 5 min. 10 μg samples were taken. β-actin was used as reference. Following 10% SDS-PAGE electrophoresis, the film was transferred and sealed, and then cultivated with GLDC antibody for 1 h at room temperature. The film was washed with PBS (with Tween-20) and further processed according to the ECL™ Chemiluminescence Kit instructions (GE Healthcare, Little Chalfont, Buckinghamshire, UK).

Cells were digested in 0.25% trypsin. A single-cell suspension liquid was made with RPMI-1640 medium with 10% fetal bovine serum. 2,000 cells were added per hole mixed with 150 μl medium, and cultivated at 37 °C with 5% CO2. Transient transfection was performed the next day, and evaluation took place after 24, 48, 72, and 96 h. To perform an MTT assay, 20 μl of 5 mg/ml MTT solution were added. A microplate reader (570 nm) determined the absorbance value of each hole.

**Statistical Analysis**
SPSS version 17.0 software (IBM Corp., Armonk, NY, USA) was used for all statistical analyses in this study. All results are shown as mean values ± standard deviation with differences between groups examined using the student’s t test. A p value less than 0.05 was considered significant; a p value less than 0.01 was considered extremely significant.

**Results**

Immunohistochemical staining performed with lymphoma tissue samples of 48 BALB/c nude mice yielded a p53 protein-positive rate of 39.6% (19/48); hence, 19 BALB/c nude mice formed the p53 protein-positive group, and 29 mice formed the p53 protein-negative group.

In the B cell lymphoma tissue of the p53 protein-positive group, GLDC mRNA expression increased to 11.32 ± 1.65 compared with 2.38 ± 0.26 in the p53 protein-negative group; the difference was significant (p < 0.01) (table 1). GLDC protein expression in the B cell lymphoma tissue of the p53 protein-positive group increased to 23.42 ± 2.26 compared with 4.36 ± 0.42 in the p53 protein-negative group (fig. 1); this difference was also significant (p < 0.01) (table 1).

In GLDC siRNA-transfected cells, GLDC protein expression was decreased by 63% (p < 0.05) (fig. 2) compared with the negative-control siRNA group and the blank control group. This showed that designed GLDC siRNA inhibits GLDC protein expression significantly, which was subsequently used to test the cells’ proliferation ability.

In the first 72 h, the cell proliferation ability in all 3 groups showed no significant differences (p > 0.05). After 72 h, the prolif-
eration ability of the GLDC siRNA-transfected cells decreased significantly compared with the negative-control siRNA group and the blank control group (p < 0.05) (fig. 3), which showed that the GLDC gene can promote tumor cell proliferation.

Fig. 1. Glycine decarboxylase (GLDC) protein expression in B cell lymphoma tissue.

Fig. 2. 24-h glycine decarboxylase (GLDC) protein expression in transfected human B lymphoma cells (Raji). 1: Blank control group; 2: negative control, siRNA group; 3: GLDC siRNA transfection group.

Fig. 3. Cell proliferation ability after GLDC siRNA transfection (MTT assay). 1: Blank control group; 2: GLDC siRNA transfection group; 3: negative control, siRNA group.

Discussion and Conclusion

Despite many advances in oncology [11, 12], clinicians still face many challenges when it comes to treating cancer. Accordingly, the treatment of lymphoma is currently still very difficult.

p53 gene mutations (mostly point mutations) represent the most common changes in human tumors. Mutant p53 protein has a longer biological half-life and increased stability, and can accumulate in cells and be overexpressed. p53 protein can be easily detected by immunohistochemistry. p53 protein encoded by wild-type p53 has a very short half-life and is present at a very low level in normal tissue cells; it cannot be detected by immunohistochemical techniques. Hence, p53 protein traced by immunohistochemistry and Western blot in tissue cells is always of the mutant type with its long half-life and strong stability [13]. Mutant p53 protein has lost its inhibitory effects on tumor cells, and p53 mutations are common in B cell lymphoma [3]. Regardless, the contribution of this p53 property to tumor suppression is not clear.

Recent studies have led to an emerging appreciation of the importance of GLDC [7]. GLDC is overexpressed in NSCLC. It is a key component of the highly conserved glycine cleavage system in the amino acid metabolism that catalyzes the breakdown of glycine to form CO₂, NH₃ and NH₂, and 10-methylene tetrahydrofolate (CH₂-THF) to fuel one-carbon metabolism [14].

To examine whether p53 mutations influence GLDC expression in B cell lymphoma, we established a B cell lymphoma animal model. Among 48 BALB/c nude mice, immunohistochemical staining of lymphoma tissue revealed a p53 protein-positive rate of 39.6% (19/48). Our study showed that both GLDC mRNA and protein expression in B cell lymphoma tissue significantly increased in the p53 protein-positive group compared with the p53 protein-negative group. The p53 protein-positive group harbors p53 gene mutations, and our experiment showed that GLDC is a gene directly regulated by the transactivation function of mutated p53.

To delineate the relationship between p53 gene mutations in B cell lymphoma murine cells and GLDC, we designed a GLDC-specific interference fragment siRNA-transfected human Raji B lymphoma cell line. In GLDC siRNA-transfected cells, GLDC protein expression decreased by 63% (p < 0.05) (fig. 2) compared with the negative-control siRNA group and the blank control group, which showed that designed GLDC siRNA inhibits GLDC protein expression significantly. Also, the proliferation ability of GLDC siRNA-transfected cells decreased significantly compared with the negative-control siRNA group and the blank control group (p < 0.05), which showed that the GLDC gene can promote cell proliferation in p53-mutated B cell lymphoma.

GLDC was found to be very important for NSCLC tumor initiating cell growth and tumor formation. Overexpression of GLDC and other glycine/serine enzymes can promote cell transformation and tumor development. GLDC induces dramatic changes in glycolysis and glycine/serine metabolism, leading to changes in pyrimidine metabolism to regulate cancer cell proliferation [7]. In humans, GLDC is part of a multienzyme complex (which includes the
lipoyl-containing H-protein) that couples the decarboxylation of glycine to the biosynthesis of serine [15].

B cell lymphoma is a set of independent diseases with strong heterogeneity, and overall a very common cancer. Our study showed that both GLDC mRNA and protein expression increased in the B cell lymphoma tissue of the p53 protein-positive group compared with the p53 protein-negative group of BALB/c nude mice. Also, the cell proliferation ability in the GLDC siRNA transfection group decreased significantly compared with the negative-control siRNA group and the blank control group (p < 0.05), which showed that the GLDC gene can promote cell proliferation in p53-mutated B cell lymphoma.

This study supports a direct relationship between p53 gene muta-
tions and GLDC expression in B cell lymphoma, p53 gene muta-
tions lead to GLDC overexpression in B cell lymphoma. GLDC can
induce dramatic changes in glycolysis and glycine/serine metabo-

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**Disclosure Statement**

The authors declare that there are no conflicts of interest.

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