Effects of Metformin on Lung Cancer Cell Proliferation and Apoptosis: An Animal Study in the Lewis Lung Cancer Model

Introduction

Lung cancer is one of the most common and lethal types of malignancy in terms of both incidence and mortality rates worldwide (1). Non-small cell lung cancer (NSCLC) accounts for approximately 85% of all lung cancer cases and is particularly notable for its potential for treatment resistance and metastasis. Current treatment options generally have limited efficacy and the need for new therapeutic approaches continues (2-4). Energy metabolism and apoptotic responses of cancer cells play critical roles in the progression of this disease and these processes are considered as therapeutic targets (5).

Although metformin is a biguanide derivative commonly used in the treatment of type 2 diabetes, it has recently attracted attention for its potential effects in cancer therapy. Metformin regulates cellular energy metabolism by activating the AMP-activated protein kinase (AMPK) pathway and reduces cellular proliferation by suppressing mTOR signaling. In addition, metformin has been shown to reduce oxidative stress, support apoptotic processes and contribute to immunological regulation in the tumor microenvironment (6,7).

In this study, the effects of metformin on lung cancer cell proliferation and its potential to activate apoptotic mechanisms were investigated using a Lewis lung cancer animal model. The study aims to evaluate both the direct effects of metformin on tumor cells and its contribution to systemic metabolic changes and to reveal its potential translational value in cancer therapy.

Method

Animal Model and Ethical Approval

Specific pathogen-free (SPF) male BALB/c mice, 6-8 weeks old, were used in the study. Mice were housed at a temperature of 22 ± 2°C and humidity of 50 ± 10% in an environment with a constant 12 h light/12 h dark cycle. All animals were maintained on a standardized diet with free access to drinking water. The study protocol was approved by the Animal Experiments Ethics Committee.

Cell Culture and Tumor Model

Lewis Lung Cancer (LLC) cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and 1% L-glutamine. Cells were incubated at 37°C in a 5% CO_2 atmosphere. Cells used for the experiment were in logarithmic phase and viability was confirmed to be above 95% by trypan blue method. LLC cells were prepared as $1x10^6$ cells/ml and 100 µl was injected subcutaneously under the right forelimb of mice.

Experimental Groups

Mice were randomly divided into three groups:

1. **Control Group (n=10):** Tumor was induced but no treatment was administered.
2. **Metformin Group (n=10):** 250 mg/kg metformin was added to drinking water and given for 21 days.
3. **Combination Group (n=10):** 250 mg/kg metformin and 2 mg/kg cisplatin (weekly intraperitoneal injection).

Treatment and Monitoring Protocol

Metformin treatment was started 7 days after tumor implantation and continued for 21 days. Cisplatin was administered intraperitoneally on days 7, 14 and 21. Mice were monitored daily and body weights and behavioral parameters were recorded. Tumor volume was determined by a calculation method based on length and width values measured weekly using a digital caliper. For this calculation, the length and width of the tumor were measured, then the length value was multiplied by the square of the width value and the result was divided by two. This method is a widely used approach to accurately and consistently assess tumor volume. For example, when the tumor length was measured as 10 mm and the width as 8 mm, the tumor volume was calculated to be approximately 320 mm^3.

Histological and Molecular Analyses

- **Histopathological Evaluation:** Tumor tissues were fixed with 10% neutral buffered formalin, embedded in paraffin and examined by hematoxylin-eosin (H&E) staining.
- **Apoptosis Analysis:** Cleaved caspase-3 expression was evaluated by immunohistochemistry.

- **Protein Analysis:** Proteins isolated from tumor tissues were analyzed by Western blotting. AMPK, p-AMPK and mTOR protein expression levels were analyzed.

Oxidative Stress and Antioxidant Activity

Malondialdehyde (MDA) levels were measured and glutathione (GSH) levels were analyzed with ELISA kits to evaluate oxidative stress in tumor tissues. Reactive oxygen species (ROS) levels were determined using DCFDA fluorescent probe.

Statistical Analysis

All data obtained from the study were analyzed using IBM SPSS Statistics (Version 25.0) software. Data were expressed as mean ± standard error of the mean (SEM) and Shapiro-Wilk test was applied to determine whether the data were normally distributed. Parametric tests were used for normally distributed data and nonparametric tests were used for non-normally distributed data. One-way analysis of variance (ANOVA) was applied to evaluate the differences between the groups. If a significant difference was detected in the ANOVA results, Tukey post-hoc test was used to determine which groups were statistically significant. For data that did not show normal distribution, Kruskal-Wallis test was applied and post-hoc analysis was performed with Dunn's test. Kaplan-Meier method was used to evaluate survival rates. The log-rank test was applied to test the difference in survival curves between the groups. Pearson correlation analysis was performed to examine the effect of metformin treatment on tumor volume and apoptosis markers. This analysis was performed to determine possible linear relationships between metformin and tumor growth. In all analyses, a value of $p<0.05p < 0.05p<0.05$ was considered statistically significant. All analyses were reviewed twice and calculations were checked to increase the reliability of the data.

References

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