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The Effect of Agrimol B on A549 Lung Cancer Cells by Blocking Mitochondria Biogenesis

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Abstract:

Purpose: Since Agrimol B can combine with PGC-1α, impair mitochondrial biogenesis, and promote tumor apoptosis, thus inhibiting colon carcinoma progression, I predict that increasing concentrations and treatment durations with Agrimol B kills A549 lung cancer cells by blocking mitochondria biogenesis.

Methods: The study will measure cell killing by MTT assay, cell migration inhibition by Boyden chamber assay, and killing of cancer cells in vivo by measuring tumor size in A549 xenograft mice and confocal microscopy with mitotracker stain to measure reduction in mitochondria. The positive control is Anlotinib Hydrochloride. The negative control is DMSO/PBS.

Possible results: There are three main possible results: (1) Agrimol B kills A549 cancer cells. It reduces mitochondria of A549 lung cancer cells, inhibits cell migration, and kills A549 lung cancer cells (2) Agrimol B promotes A549 cancer cells. It promotes mitochondria of A549 lung cancer cells and cell migration. (3) Agrimol B does not significantly affect the mitochondria biogenesis and migration of A549 cancer cells.

Conclusion: The result of the study will provide important insight into the effect of Agrimol B on A549 cancer cells. It would increase our understanding of the treatment of cancer with Chinese medicine at the molecular level and improve human health.

Keywords: Agrimol B, PGC-1α, lung cancer.

1. Introduction

1.1 Agrimol B

Agrimonia pilosa Ledeb. It is an herbal medicine recorded in Atlas of Materia Medica. [1] It has been proven to resist cancer. Agrimol B ($C_{37}H_{46}O_{12}$) is a chemical in Agrimonia Pilosa. It has a molecular weight of 682.8 g/mol. According to previous research, Agrimol B can combine with PGC-1α, impair mitochondrial biogenesis, and promote tumor cell apoptosis, thus inhibiting colon carcinoma progression.[2] However, research on Agrimol B's inhibition of other types of cancer is limited, especially in lung cancer.

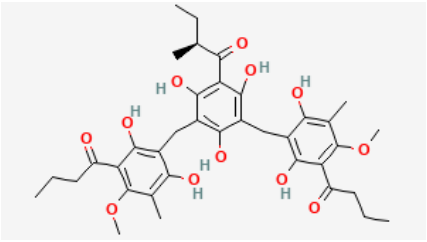

Figure 1. The structure of Agrimol B.

1.2 Lung Cancer

Lung cancer is the third most common cancer in the U.S. and China. Health systems report over 800,000 new cases of lung cancer each year in China. Non-small cell lung cancer (NSCLC) is the most common type of lung cancer. It accounts for over 80% of lung cancer cases. Common types of lung cancer include adenocarcinoma and squamous cell carcinoma. Chemotherapy is the main treatment for lung cancer, and more than 90% of lung cancers require chemotherapy treatment. The effect of chemotherapy on small cell lung cancer is relatively positive in both early and late stages, and about 1% of early small cell lung cancer is cured by chemotherapy. Chemotherapy generally does not cure non-small cell lung cancer but only prolongs patients' survival and improves quality of life. Thus, developing new drugs and exploring their pharmacological mechanisms are crucial for improving the treatment outcomes of CRC. In recent years, a wide range of research at the molecular level has been done to explore the treatment of cancer with Chinese medicine.

Peroxisome proliferator-activated receptor-γ coactivator

PGC-1α is a nuclear transcriptional coactivator in a family of transcription coactivators that plays a central role in regulating cellular energy metabolism. It is described as a master regulator of mitochondrial biogenesis and function. (PGC)-1αstimulates mitochondrial biogenesis. [3]The decreased expression of PGC-1α coactivates PPAR γ and thus reduces transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation. Since the blocking of mitochondria can affect human body cells, the negative side effects of Agrimol B are to be researched.

1.3 Hypothesis

Since Agrimol B can combine with PEC-1α, impair mitochondrial biogenesis, and promote tumor cell apoptosis, thus inhibiting colon carcinoma progression, I predict that increasing concentrations and treatment durations with Agrimol B kills and decreasing migration of A549 lung cancer cells in vitro and decreasing tumor size in xenograft.

2. Methods

2.1 Positive controls, negative controls, and experimental groups

In measuring the cell killing rate with MTT assay, measuring the cell migration with Boyden chamber assay, and measuring reduction in mitochondria with confocal microscopy, the positive control is cells treated with 100nM Anlotinib Hydrochloride, which is a chemical proven to kill lung cancer cells and which is used in medicine to treat lung cancer. The negative control is cells treated with 1% DMSO. There are three experimental groups, each treated with different concentrations, which are 0, 100, and 300nM of Agrimol B. Measurement will be taken after 6, 12, and 24 hours of treatment.

For measuring the tumor size in vivo experiment, the positive control is the group in which A549 xenograft mice are treated daily with 100nM Anlotinib Hydrochloride in their drinking water. The negative group is mice fed with normal pure water.

30 A549 xenograft mice are divided into two groups of 15. 15 are treated with various concentrations of Agrimol B in their drinking water for 1/2/4/7/14 days. 15 are the control being fed with normal pure water. A549 tumor size was measured. All the treatment groups are compared with the control group, which receives no treatment.

2.2 Toxins

Agrimol B needs to be prepared. In 50% methanol, a 0.5 mg/ml stock solution of Agrimol B was produced.

2.3 Animals

A549 xenograft mice 2 months- of age weighing 20-23g are obtained from the laboratory.

2.4 Cell Culture

A549 lung cancer cells are grown in a 96-well microplate (200 l aliquots each well) and incubated in a CO2 incubator under strict conditions (5 percent CO2, 37°C, and 95% humidity). For the following experimental procedures, each culture was generated in triplicate.

Cells were subjected to 0, 100, and 300 nM of Agrimol B supplied at the beginning (0h) and after 24 hours of the culture to evaluate the influence of Agrimol B on the mitochondrial activity of A549 lung cancer cells.

2.5 Cell killing: MTT assay

A549 lung cancer cells were seeded into 12-well microtiter plates at a density of 60,000 cells per well. 24 hours after incubation at 37°C in 5% CO2. A positive control (cell growth media with 100nM Anlotinib Hydrochloride) and a negative control (cell growth media with DMSO) were included in every experiment. The cells were harvested at the end of the exposure, and the cell killing was assessed using an MTT assay. The absorbency of light (wavelength 490nm) of each microtiter plate is measured with MTT assay. All the treatment groups are compared with the control group, which receives no treatment. The cell-killing effect is calculated through colorimetric determination.[4]

2.6 Boyden chamber assay for cell migration

Use Bayden chamber assay with membrane pore size with membrane pore size of 10μm. Treat A549 lung cancer cells with 0, 100, and 300 nM of Agrimol B, the positive control with 100 nM Anlotinib Hydrochloride, and the negative control with DMSO. After six hours of cell growth at 37°C in 5% CO2, measure the cell migration of each group. The result of each group is compared with that of the control groups.

2.7 Reduction in mitochondria: confocal microscopy

Treat A549 lung cancer cells with 0, 100, and 300 nM of Agrimol B, the positive control with 100 nM Anlotinib Hydrochloride, and the negative control with DMSO. Apply mitochondrial stain to each group of cells and measure the number of mitochondria before the treatment. After 24 hours of cell growth at 37°C in 5% CO2 under the treatment. Then, measure the reduction of mitochondria of each group of cells with confocal microscopy. The result of each group is compared with that of the control groups.

2.8 In vivo experiment

30 A549 xenograft mice are divided into two groups of

15. 15 are treated with various concentrations of Agrimol B (0, 100, 300 nM) in their drinking water for 12 hours, 1/2/4/7/14 days. 15 are the control being fed with normal pure water. A549 tumor size was measured. All the treatment groups are compared with the control group, which receives no treatment.

3. Possible Results

Table 1. Possible combination of results.

Possible observations	CR1	CR2	CR3	CR4	CR5	CR6	CR7	CR8	CR9	CR10	CR11	CR12	CR13	CR14	CR15	CR16
A549 cell killed?	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Cell migration rate decrease?	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
Does the number of active mitochondria decrease?	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
Size of tumor decrease?	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Supporting hypothesis?	Yes	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	No

Note. "+" represents that the answer to the question in the chart is "yes". Compared to the control group, the cellular activity decreases, the cell migration rate decreases, the number of active mitochondria decreases, or the tumor size decreases "-" represents that the answer to the question in the chart is "no". Compared to the control group, the cellular activity does not decrease, the cell migration rate does not decrease, the number of active mitochondria does not decrease, and the size of the tumor does not decrease.

3.1 Possible results related to concentration change

For each combination of possible results, compared to the control groups, the effect of Agrimol B on the experimental groups may increase, decrease, or remain the same.

3.2 Possible results related to treatment durations

For each combination of possible results, compared to the control groups, the effect of Agrimol B on the experimental groups may increase, decrease, or remain the same.

4. Discussion

4.1 Possible Result 1

A549 cells are killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria decreases. The size of the tumor decreases. This result fully supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation, resulting in killed cells, decreased cell migration rate, decreased number of active mitochondria and decreased size of tumor.[5] The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. [6]

4.2 Possible Result 2

A549 cells are killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria decreases. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation, resulting in killed cells, decreased cell migration rate, decreased number of active mitochondria and decreased size of tumor. However, other factors may affect the combination of Agrimol B with PGC-α. The result means that Agrimol B inhibits the cellular activity of A549 cells and does not control tumor growth. Further experiments should elimi-

nate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth.

4.3 Possible Result 3

A549 cells are killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria does not decrease. The size of the tumor decreases. This result partially supports the hypothesis. The result may be that Agrimol B does not cause PGC-1 inactivity. However, it reduces transcription of metabolic genes, leading to decreased oxidative phosphorylation, resulting in killed cells, decreased cell migration rate, and decreased size of tumor through other transduction pathways. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should find out factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth.

4.4 Possible Result 4

A549 cells are killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria does not decrease. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be that Agrimol B does not cause PGC-1 inactivity. However, it reduces transcription of metabolic genes, leading to decreased oxidative phosphorylation, resulting in killed cells and decreased cell migration rate. The result means that Agrimol B inhibits the cellular activity of A549 cells. However, it does not control tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. Further experiments should also discover the transduction pathway contributing to A549 cell inhibition.

4.5 Possible Result 5

A549 cells are killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria does not decrease. The size of the tumor decreases. The result may be that Agrimol B does not cause PGC-1 inactivity. However, it reduces transcription of metabolic genes, leading to decreased oxidative phosphorylation, resulting in killed cells and decreased cell migration rate. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should determine the transduction pathway contributing to A549 cell inhibition.

4.6 Possible Result 6

A549 cells are killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria decreases. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation, resulting in killed cells, decreased cell migration rate, decreased number of active mitochondria and decreased size of tumor. However, other factors control the migration of A549 cells. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. Further experiments should also discover the factors contributing to the migration of A549 cells.

4.7 Possible Result 7

A549 cells are killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria does not decrease. The size of the tumor decreases. This result partially supports the hypothesis. The result may be that Agrimol B does not cause PGC-1 inactivity. However, it reduces transcription of metabolic genes, leading to decreased oxidative phosphorylation, resulting in killed cells and decreased tumor size. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. Further experiments should also discover the factors contributing to the migration of A549 cells.

4.8 Possible Result 8

A549 cells are killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria does not decrease. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be that Agrimol B does not cause PGC-1 inactivity. However, it reduces transcription of metabolic genes, leading to decreased oxidative phosphorylation, resulting in killed cells and decreased size of tumor. Other factors exist that prevent Agrimol B from inhibiting tumor growth. The result means that Agrimol B inhibits the cellular activity of A549 cells. Further experiments should determine the factor contributing to the migration of A549 cells.

4.9. Possible Result 9

A549 cells are not killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria decreases. The size of the tumor decreases. This result

partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation, resulting in decreased cell migration rate, decreased number of active mitochondria and decreased size of tumor. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth.

4.10. Possible Result 10

A549 cells are not killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria decreases. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation and decreased cell migration rate. However, other factors exist that prevent Agrimol B from inhibiting tumor growth. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth.

4.11. Possible Result 11

A549 cells are not killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria does not decrease. The size of the tumor decreases. This result partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation, resulting in decreased cell migration rate, decreased number of active mitochondria and decreased size of tumor. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. Further experiments should also explore the factors contributing to tumor growth inhibition in vivo.

4.12. Possible results 12

A549 cells are not killed by Agrimol B. Cell migration rate decreases. The number of active mitochondria does not decrease. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be that Agrimol B does not cause PGC-1 inactivity. However, it reduces the transcription of metabolic genes, leading to a decreased migration rate. Other factors exist that prevent Agrimol B from inhibiting tumor growth. The result means that Agrimol B inhibits the cellular activity of A549 cells. Further experiments should determine the factor contributing to the migration of A549 cells. Further experiments should also focus on other factors that may prevent Agrimol B from functioning in vivo.

4.13. Possible results 13

A549 cells are not killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria decreases. The size of the tumor decreases. This result partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation and decreased cell migration rate. However, other factors affect cell migration rates. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. Further research should also expand on factors that affect the migration rate of A549 cells.

4.14. Possible results 14

A549 cells are not killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria decreases. The size of the tumor does not decrease. This result partially supports the hypothesis. The result may be due to the inactivity of PGC-1α, which leads to decreased expression of PGC1, thus coactivating PPAR γ and reducing transcription of metabolic and mitochondrial genes, leading to decreased oxidative phosphorylation and decreased cell migration rate. However, other factors affect cell migration rates. The result means that Agrimol B inhibits the cellular activity of A549 cells and controls tumor growth. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth. Further research should also expand on factors that affect the migration rate of A549 cells.

4.15. Possible results 15

A549 cells are not killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria does not decrease. The size of the tumor decreases. This result partially supports the hypothesis. The result may be due to the fact that Agrimol does not cause inactivity of

PGC-1α, but it leads to the decreased size of the tumor. The result means that Agrimol B only inhibits the cellular activity of A549 cells in vivo and controls tumor growth. Further experiments should explore the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth.

4.16. Possible results 16

A549 cells are not killed by Agrimol B. Cell migration rate does not decrease. The number of active mitochondria does not decrease. The size of the tumor does not decrease. This result does not support the hypothesis. The result may be that Agrimol B does not significantly inhibit the cellular activity of A549 cells, which may be due to other factors that disrupt the inhibition of Agrimol B to A549 cells. Further experiments should eliminate the effect of factors other than the inactivity of PGC-1α that can contribute to the inhibition of A549 cells and restriction of tumor growth.

4.17. Possible results related to concentration change

For each combination of possible results, compared to the control groups, the inhibiting effect of Agrimol B on the experimental groups may increase, decrease, or remain the same. If the inhibiting effect of Agrimol B increases as the concentration increases, Agrimol B is proven to inhibit A549 cell growth. If the inhibiting effect of Agrimol B decreases as concentration increases, Agrimol B is proven to promote A549 cell growth. If the inhibiting effect of Agrimol B remains the same, Agrimol B does not significantly influence A549 cell growth.

4.18. Possible results related to treatment durations

For each combination of possible results, compared to the control groups, the effect of Agrimol B on the experimental groups may increase, decrease, or remain the same. If the inhibiting effect of Agrimol B increases as duration increases, Agrimol B is proven to inhibit A549 cell growth. If the inhibiting effect of Agrimol B decreases as duration increases, Agrimol B is proven to promote A549 cell growth. If the inhibiting effect of Agrimol B remains the same, Agrimol B does not significantly influence A549 cell growth.[7]

5. Conclusion

In conclusion, this study explores the effect of Agrimol B on human A549 lung cancer cells and in mice. The result of the study would indicate whether Agrimol B can inhibit cell growth, migration, and tumor formation in vitro and in vivo. Further research is expected to focus on the side effects of Agrimol B on human body cells.

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