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The Increase of the Senescence in Human Lung Adenocarcinoma Cell Line A549 by Tripterygium, A Diterpenoid from Chinese Traditional Medical Herb

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Abstract: Since using andrographolide to increase senescence in human lung requires extremely high concentration on the andrographolide. Therefore, we want to find another molecules in TCM that also increase the senescence in human lung adenocarcinoma. I am going to measure senescence by B-Gal assay, and p21 protein abundance by western blot, and p53 activity by p53 reporter assay. Positive control is MLN4924 and negative control is DMSO/PBS. Here lists three major possible results. 1.Tripterygium can increase both p53 and p21 so that the senescence in human lung adenocarcinoma can be increased. 2 Tripterygium can only increase either p53 or p21 so that the senescence cannot be increased. 3. Triptergium can only increase either p53 or p21 that can increase the senescence. We aim to determine the efficacy of Tripterygium to increase the senescence in human lung cancer cell.

Keywords: Chinese Traditional Medicine, Lung cancer, Tripterygium, Increasing senescence

1. Introduction

Nowadays, as the increasing number of people who smoke, it is more likely that people could suffer from lung cancer by smoking or passive smoking. Lung cancer is a harsh public health challenge in cancer aspect. Despite surgical interventions, patient survival are still approximately 50% mortality within 5 years across early stages1.

Therefore, chemotherapy and immunotherapy methods could be a better remedy for lung therapy. Since using andrographolide to increase senescence in human lung requires extremely high concentration on the andrographolide which is not practical for Chemotherapy2, I wonder if there is another molecules in Chinese traditional herb medicine could also increase the senescence in human lung and be practical for chemotherapy.

Triptolide is a diterpene triepoxide which is a major component of extracts derived from the medicinal plant Tripterygium wilfordii Hook F (TWHF).3 Triptolide medical activities are various, for instance, anti-inflammation, modulating immune system and proapoptotic activity. So, triptolide has been widely used to treat inflammatory diseases, autoimmune diseases, organ transplantation and even tumors. Triptolide can not only increase tumor cell apoptosis directly, but can also enhance apoptosis increased by cytotoxic agents such as TNF-α, TRAIL and chemotherapeutic agents regardless of p53 phenotype by inhibiting NFκB activation. The targets of triptolide have been recently demonstrated such as HSP, MKP-1,5-Lox, and histone methyl-transferases.4

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Tumor protein P53, which is also called transformation-related protein 53 (TRP53) is a regulatory protein that is extremely easy to be mutated in human. Which is a vital factor of cancer being triggered in a pathway. The p53 proteins are effective in human body to prevent cancer formation.5

P21 protein is a strongly CDK inhibitor (CKI). It can bind to and inhibit the activity of cyclin-CDK2, -CDK1, and -CDK4/6 complexes. In other words, therefore, it plays a role as a regulator of cell cycle at the phase between G1 and S. The binding of p21 to CDK complexes is homologous to the other CIP/KIP CDK inhibitors p27 and p57. Specifically it contains a Cy1 motif in the N-terminal half, and weaker Cy2 motif in the C-terminal domain that allow it to bind CDK in a region that blocks its ability to complex with cyclins and thus prevent CDK activation.6

p21 is a protein that is activated by the transcription factor p53. When p53 is activated, often due to stress signals like DNA damage, it increases the expression of the p21 gene. The p21 protein then acts as a cyclin-dependent kinase inhibitor, preventing the phosphorylation of RB, which helps maintain cell cycle arrest. This relationship is crucial for controlling cell division and preventing tumor growth.

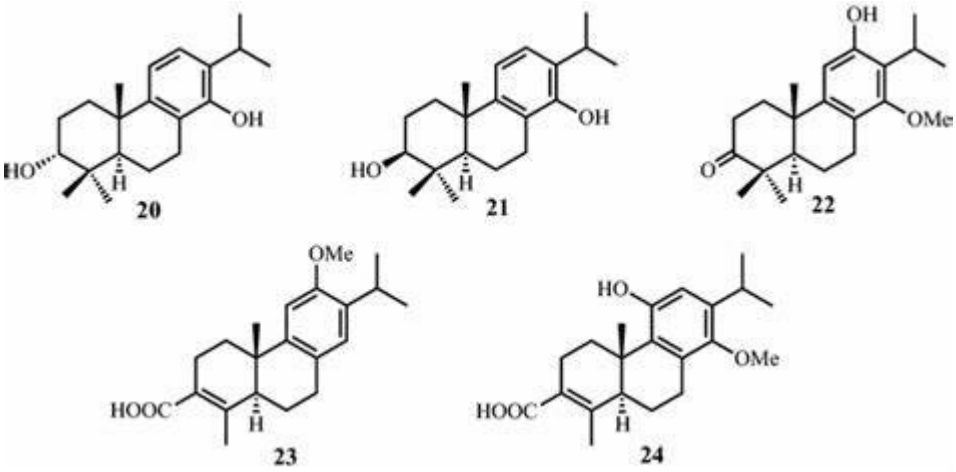

Figure 1: Diterpenoids from tripterygium

Hypothesis:

I predict that increasing concentrations and treatment durations with Triptolide from Chinese Traditional medicinal herb “Tripterygium” increases p53 and p21 protein which leads to a senescene in human lung adenocarcinoma cell line A549.

2. Material and methods

2.1. Western blot

To examine p21 protein abundance using Western blot, specific equipment such as a gel electrophoresis system, transfer apparatus, incubator, and imaging system are needed. Use MLN4924 as a positive control, typically at 1 μM for around 24 hours, to increase p21 expression. The negative control should be DMSO/PBS, using the same concentration of DMSO as in the experimental treatment. After treatment, lyse the cells using a buffer containing protease inhibitors, and quantify protein concentration using a BCA or Bradford assay. Load equal amounts of protein (20-30 μg) onto SDS-PAGE gels and perform electrophoresis to separate the proteins by size.

Transfer the separated proteins to a nitrocellulose or PVDF membrane. Block the membrane with 5% non-fat milk or BSA in TBST for 1 hour at room temperature to prevent non-specific binding. Incubate the membrane with a primary antibody against p21, typically diluted 1:1000 in blocking

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buffer, for 1-2 hours at room temperature or overnight at 4°C. After washing, incubate with a secondary antibody, diluted 1:5000, conjugated with HRP or a fluorescent tag, for 1 hour at room temperature. Wash the membrane thoroughly and apply a chemiluminescent substrate for detection if using HRP-conjugated antibodies. Capture the images using an imaging system.

Include a loading control, such as GAPDH or β-actin, to ensure equal protein loading across samples.

2.2. B-Gal assay

To measure senescence in lung cancer cells using the β-Galactosidase (B-Gal) assay, begin by culturing the cells under appropriate conditions until they reach the desired confluence. Firstly, treat the cancer cell with MLN4924 and DMSO/PBS and Triptergium. Then, put the cells into PBS solution (phosphate-buffered saline) in order to remove any residual. Using fixative solution to fix the cell, such as 2% formaldehyde and 0.2% glutaraldehyde, for 10-15 minutes at room temperature. After fixing, wash the cells again with PBS to remove the fixative. Prepare the staining solution by mixing 1 mg/mL of X-gal in a buffer containing potassium ferricyanide, potassium ferrocyanide, and magnesium chloride at pH 6.0, which is optimal for SA-β-Gal activity. Add the staining solution to the cells and incubate them at 37°C without CO2 overnight. The presence of senescent cells will be indicated by blue staining, as the X-gal substrate reacts with SA-β-Gal. Finally, analyze the cells under a microscope, counting the number of blue-stained cells to determine the percentage of senescent cells in the population. This method provides a reliable means to assess the extent of cellular senescence in lung cancer research.

2.3. Reporter assay

To measure the abundance of p53 in cells using a reporter assay, firstly, we transfect the cells with a plasmid containing a reporter gene, under the control of MLN4924 and DMSO/PBS. Allow the cells to express the reporter gene under appropriate culture conditions. After the culturing period, typically 24-48 hours, we lyse the cells to release the reporter enzyme. Add a substrate specific to the reporter enzyme, such as luciferin for luciferase, and measure the luminescence using a luminometer. The luminescent signal intensity correlates with the activity of p53, as it drives the expression of the reporter gene. This assay provides a quantitative measure of p53 activity, allowing researchers to assess its abundance and functionality in various experimental conditions.

2.4. Statistical analysis

The experiments should be finished twice. Students T- test and ANOVA are used for analyze the data.

3. Results

Table 1: Possible results

Combination Result # (CR#)				
	Trip increases p53 by reporter assay	Trip increases p21 by western blot	Trip increases senescence by b-gal assay	Support of hypothesis
1	+	+	+	Full
2	+	+	-	Partial
3	+	-	+	Partial
4	-	+	+	Partial

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Table 1: (continued)

5	-	-	+	Partial
6	-	+	-	Partial
7	-	-	+	Partial
8	-	-	-	Fully Contradicts

Table legend: "+" indicates Tripterygium affects the senescence similarly like MLN4924 the, "-" indicates Tripterygium affects the senescence similar like the DMSO/PBS.

Tripterygium can increase the p21 leading to the increase in the p53

Possible result 1:

Tripterygium can increase the p21 which will cause the p53 to start replication. Then, the these p53 will increase the senescence in lung cancer after the CDK p21 being increased. Therefore, this result fully supports my hypothesis.

Possible result 2:

Tripterygium can increase the p21 which will cause the p53 to start replication. However, the replication of the p53 does not inactivate the cell line A549 to increase senescence.

Possible result 3: Tripterygium can directly increase the p53 replication leading to the increases of senescence.

Tripterygium can increase the p21, but the p21 cannot be a CDK for the replication of p53

Possible result 4:

Tripterygium increase p21 replication but p53 is not affected by the CDK p21. However, p21 directly make contribution to the increases of senescence of lung cancer.

Possible result 6: Tripterygium increase p21 replication but p53 is not affected by the CDK p21. Therefore, the p53 cannot make increase in the senescence of lung cancer.

Possible result 5: **Tripterygium can directly increase the senescence without activating any DNA to increase p21 or p53**

Possible result 8: **Tripterygium cannot activate p21 or p53, thus no senescence is increased.**

4. Discussion

Possible result 1:

This result fully support my hypothesis. Tripterygium can successfully activate the p21 protein by either make its inhibitor such as Akt lose its function or directly activate p21 and p21 can be directly used for the start of replication of p53. Therefore, after the abundance of p53 being increased, the cell line A549 will be inactivated or even shut down leading to the senescence in lung cancer adenocarcinoma.

Possible result 2:

This result partially support my hypothesis. Although Triptergium can successfully activate p21 that leads to replication of p53. The cell line A549 may also being affected by tripterygium. For instance, A549 being enhanced by this molecule.

For another possible reason, the p51 might be mutated, denatured or changed in structure since th e presence of tripterygium. Thus, there are no obvious increase in senescence in the lung adenocarci noma.

Possible result 3: This result partially support my hypothesis. Tripterygium cannot interact with the p21 protein. Nonetheless, it perhaps could affect another CDK of p53 to increases the replication of p53 such as CDK 4/6. Or Tripterygium posses the function to directly increase the replication of p53. Which could finally leads to the shut down of cell line A549

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For another possible reason, the tripterygium can make the inhibitor of the p53 such as CKl1 lose its function.

Possible result 4: This result partially support my hypothesis. Tripterygium can either make p21's inhibitor such as Akt lose its function or directly activate p21. And it might change the structure of p21 which can lead p21 directly inhibit the cell line A549.

For another factor, the tripterygium can both activate p21 and the cell line A549, for instance, activating another signaling pathway that could increase the senescence in A549.

Possible result 5:

This result partially support my hypothesis. Tripterygium can only activate A549 by activating signaling pathway that could increase the senescence in A549. Or it could activate other CDK or enzymes that could increase the cell line A549.

Possible Result 6: This result partially support my hypothesis. Tripterygium increase p21 replication but p53 is not affected by the CDK p21, either changing the function of p21 or having the funtion to inhibit p53. Therefore, the p53 are not replicated, meaning no increase in the senescence of lung cancer.

PR8: This result fully contradicts to my hypothesis. **Tripterygium cannot activate p21 or p53, cell line A549 cannot be activated, thus no senescence is increased.**

5. Conclusion

The study aim to find out whether the Tripterygium can be an effective substitutes for andrographolide to increase senescence in human lung cancer. We use Western Blot to observe the change in amount of p21, Reporter assay to examine whether there is a increase in p53 and B-Gal assay to observe senescence in lung cancer after treating cancer cell with tripterygium. We analysis the possible results based on the CDK of p21 or p51 and the potential enzymes that could inhibit the function of p21 or p53 being activate by tripterygium and whether Tripterygium can be used for activate the cell line A549. Experiments should be done in the future to test either the efficacy of Tripterygium or the validity of our analysis for the possible results. If the experiment does support my hypothesis, we could have more choices of treatment with human lung cancer.

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