

Can SDH1 in Chinese Medicine Target ALK for NSCLC Remission?

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Abstract. Purpose: This study aims to investigate whether SDH1, the active ingredient of the Chinese medicine compound *Selaginella Doederleinii* Hieron, can effectively inhibit anaplastic lymphoma kinase (ALK) activity in non-small cell lung cancer (NSCLC). NSCLC is a significant and challenging disease, which has limited treatment options with widely existing adverse events (AEs). The research will involve both in vitro and in vivo experiments to assess the potential of SDH1 to target and restrain ALK kinase activity, potentially leading to the remission of NSCLC. **Methods:** Materials for this study included human NSCLC cell lines cultured in specific conditions and various reagents. In vitro, the level of protein is pressured by Western blot, and cell growth is assessed by MTT assay. In vivo, experiments used a xenograft tumor mouse model injected with NSCLC cells and treated with different reagents, with tumor growth and other observations recorded. **Possible Results:** There are three most possible results: (1)SDH1 can inhibit ALK kinase to control the growth of NSCLC cells; (2) SDH1 cannot target ALK kinase but it can control the growth of NSCLC cells; (3) SDH1 cannot restrain the activity of ALK kinase neither in vitro nor in vivo, thus it cannot kill NSCLC cells. **Conclusion:** The result of our study investigates the impact of SDH1 on anaplastic lymphoma kinase (ALK) and A549 NSCLC. The findings could reveal the potential of targeting ALK to induce remission in non-small cell lung cancer both in laboratory and animal models, which tends to provide a possible avenue for future clinical and pharmaceutical approaches to combat NSCLC.

Keywords: Chinese medicine, Anaplastic lymphoma kinase, Non-small cell lung cancer.

1. Introduction

Globally, lung cancer is the most common and lethal cancer [1]. It is also the primary cause of cancer-related deaths. For stage IV lung cancer, palliative surgery, chemotherapy, radiation, and targeted therapy are the standard treatment approaches. Without appropriate treatment, however, the median survival time for individuals with advanced lung cancer was only 3.3 months [2]. Small-cell lung cancer and non-small cell lung cancer (NSCLC) are the two forms of lung cancer. Of all lung malignancies, NSCLC is the most frequent, making up 85% of cases [3]. Due to its specificity and effectiveness, molecularly targeted therapy has shown to be a wise decision for certain lung cancer patients in recent years [2]. Nevertheless, not all forms of non-small cell lung cancer can benefit from them, and certain treatments may have unfavorable side effects [4] and significant financial costs [5]. Thus, there is a great need to investigate safer and more effective therapeutic approaches that target specific NSCLC receptors.

Of the known receptors targeted by non-small cell lung cancer (NSCLC), a rearrangement in the gene anaplastic lymphoma kinase (ALK) is present in approximately 5% of NSCLCs. ALK is a member of the insulin receptor superfamily and has the closest genetic link to another receptor protein-tyrosine kinase called leukocyte tyrosine kinase (Ltk) [6]. The aberrant ALK protein produced by the ALK gene rearrangement leads to the growth and division of the cells [4]. Receptor tyrosine kinase ALK protein rearrangements lead to signal dysregulation in the kinase domain of non-small cell lung cancer (NSCLC) [7]. Twenty different ALK-fusion proteins resulting from different chromosomal rearrangements have been reported, and these are linked to the onset of different illnesses. EML4-ALK and four other forms of these fusion proteins are important in about 5% of non-small cell lung cancers [6]. In addition to cases of ALK overexpression, childhood neuroblastomas are associated with almost twenty ALK activating mutations [6]. Tumor development is significantly influenced by the activation of the ALK protein kinase domain, which is brought about via dimer formation involving the amino-terminal region of ALK fusion proteins [6]. The cell proliferation module made up of Ras/Raf/MEK/ERK1/2 and the cell survival route including JAK/STAT are two of the signaling pathways that follow ALK fusion proteins [6]. Since ALK is a protein that is translated from the ALK gene, changes in the ALK gene might cause the ALK protein to become overactive, which may simultaneously encourage cell division and possibly aid in the emergence of some malignancies [7]. Therefore, individuals with anaplastic lymphoma kinase (ALK)-positive non-small cell lung cancer (NSCLC) may induce remission by inhibiting the activity of ALK protein.

The side effects of Alk inhibition are expected to vary from person to person. According to previous research, nearly all participants experienced adverse events (AEs) while using ALK inhibitors, and more than 20% of them encountered serious adverse events (SAEs). In the case of ceritinib and brigatinib, SAEs affected over 40% of participants. Overall, ALK inhibitors exhibited considerable lung-related side effects [8]. Besides, traditional medicine might cause the human body to produce drug resistance. For instance, crizotinib (known Alk inhibitor) drug resistance typically develops around 10 months after starting the treatment and around 28% of crizotinib resistance cases can be attributed to nearly a dozen different mutations in the ALK gene within the EML4-ALK fusion protein [6].

In previous research, a Chinese medicine called *Selaginella Doederleinii* Hieron has been proven to have significant efficacy on stage IV lung adenocarcinoma patients. Its major ingredient is (E)-hordenine-[6-O-(4-hydroxycinnamoyl)- β -D-glucopyranosyl]- α -L-rhamnopyranoside (SDH1) which has 14 targets in cells [2]. In another word, SDH1, the active ingredient, is expected to block cell growth by restraining the activity of ALK kinase, which is related to cell growth, and might cause remission of non-small cell lung cancer (NSCLC).

Based on some research done by other research groups, there are still some questions that need to be solved, like the undesirable side effects [4] and the high economic burden [5] of traditional therapies. So my research question is whether SDH1 can target anaplastic lymphoma kinase (ALK) and restrain its activity to cause remission of non-small cell lung cancer. Therefore, to test the effect of molecularly targeted therapy of SDH1, a further study should be designed. This paper intends to investigate the effect of using SDH1 to restrain the activity of ALK protein, both in vitro and in vivo conditions.

Hypothesis: I predict that increasing concentrations and treatment durations with SDH1 can inhibit anaplastic lymphoma kinase (ALK) specifically but not other kinase, and restrain its activity to kill A549 non-small cell lung cancer in vitro and to reduce xenograft tumor size in vivo. Measure killing by MTT assay and Alk phosphorylation by western blot. A positive control is the known Alk inhibitor Ceritinib (LDK378) and negative control is DMSO/PBS.

2. Materials and methods

2.1. Materials

2.1.1. Cell culture

Human NSCLC cell lines (supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in humidified air containing 5% CO₂) [9].

2.1.2. Groups

Notes: need pre-experiments to adjust the concentrations (include cell and reagents) to get better results and observation.

2.1.2.1. Reagent

- a. **Control: SDH1**
- b. **Positive control: Ceritinib** (Clinically used for treatment patients with anaplastic lymphoma kinase (ALK)-positive non-small cell lung cancer)
- c. **Negative control: DMSO/PBS**

2.1.2.2. Concentration

In order to obtain the relationship between concentration and efficacy of SDH1, each reagent is added at three different concentration (Low, Moderate, High).

2.1.2.3. Repeated experiment

To improve the reliability of results, each group includes 3 independent trial. The final results are the average of independent trials in each group.

2.2. Methods

2.2.1. In vitro condition

2.2.1.1. Method

- a. **Western blot** : measure Alk phosphorylation (protein concentration of ALK kinase).

Protein samples are prepared from the nucleus, whole-cells, or tissues. Protein concentration is determined with Coomassie protein assay reagent using BSA as the standard. [9]

- b. **MTT assay**: measure the survival rate of NSCLC cells (set the control group = 100%).

The MTT reagent can be metabolized into formazan by active cells [10]. It is a chromogenic nature of this redox chemical reaction that provides a colorimetric-based measurement of intracellular formazan [11]. The MTT assay is typically performed after a few hours of incubation of cells with MTT. The water-insoluble formazan produced is then solubilized by a solvent such as Dimethyl sulfoxide (DMSO). Subsequently, the lowering of the light transmission by absorbance and other mechanisms by the homogenized MTT-formazan solution is measured by a microplate reader in terms of its optical density (OD) at a wavelength at which MTT-derived formazan absorbs the most (around 570 nm). The measured OD values are assumed to be a representation of formazan concentration and consequently the intracellular reduction of MTT. [12]

2.2.1.2. Treatment duration

Data measurements were performed respectively after 1, 3, 5, 7 day of sample treatment.

2.2.2. In vivo condition: Anatomical observation

2.2.2.1. Method

Xenograft tumor mice model of human NSCLC: measure the tumor volume and whole body weight.

The 4-6 week old male nude mice are housed in specific pathogen-free (SPF) condition, and provided with sterile food and water. The mice are acclimatized for one week before being used for experiments. NSCLC cells suspend in 200 ml of PBS are injected subcutaneously into the right flank of the mice.

After one week, tumor-bearing mice are randomly divided into three groups. Three different types of reagents are administered three times a week in each mouse for 3 successive weeks via intraperitoneal injection. At the end of the experimental period, all animals are sacrificed by cervical decapitation. The tumor tissues are excised aseptically, weighed, and used for further study. [9]

2.2.2.2. Treatment duration

The tumor volume and whole body weight are measured three times a week after tumor inoculation.

2.2.3. Statistical analysis

The collected data need to be compared to other groups statistically. If P-values<0.05, the data would be considered significant statistically.

3. Possible results

3.1. Possible result for reagent groups

Table 1. The overview of Possible Results for Reagent Groups.

	Method	CR 1	CR 2	CR 3	CR 4	CR 5	CR 6	CR 7	CR 8
Decrease in protein concentration of ALK kinase	Western blot	+	+	+	-	+	-	-	-
Decrease in the number of surviving cells	MTT assay	+	+	-	+	-	+	-	-
Decrease in the volume of tumor	Anatomical observation	+	-	+	+	-	-	+	-
Conclusion (supporting Hypothesis?)		Support	Partial support	Partial support	Partial support	Partial support	Partial support	Partial support	No

Note. “+” represents a change similar to positive control. “-” represents a change similar to negative control.

Possible Result 1: Applying SDH1 can reduce the protein concentration of ALK kinase and the survival rate of Human NSCLC cell lines and in vitro. At the same time, in vivo animal models, the growth of tumor is inhibited.

Possible Result 2: Applying SDH1 can relatively reduce the protein concentration of ALK kinase and the survival rate of Human NSCLC cell lines in vitro while the growth of tumor in vivo animal models is not controlled.

Possible Result 3: Applying SDH1 can greatly reduce the protein concentration of ALK kinase while the survival rate of Human NSCLC cell lines drops not obviously in vitro. But in vivo animal models, the inhibitory effect of tumor growth is observed.

Possible Result 4: Applying SDH1 cannot reduce the protein concentration of ALK kinase. However, the survival rate of Human NSCLC cell lines in vitro decreases largely. Simultaneously, the inhibitory effect of tumor growth in vivo animal models is observed.

Possible Result 5: Applying SDH1 can reduce the protein concentration of ALK kinase while the inhibitory effect of the survival rate of Human NSCLC cell lines and tumor growth in vivo animal models is not shown.

Possible Result 6: Applying SDH1 cannot reduce the protein concentration of ALK kinase or the tumor growth in vivo animal models. However, the survival rate of Human NSCLC cell lines in vitro decreases.

Possible Result 7: Applying SDH1 cannot reduce the protein concentration of ALK kinase and the survival rate of Human NSCLC cell lines in vitro. But the tumor growth in vivo animal models can be controlled.

Possible Result 8: Applying SDH1 cannot reduce the protein concentration of ALK kinase, the survival rate of Human NSCLC cell lines in vitro or the tumor growth in vivo animal models.

3.2. Possible results for concentration groups

Possible Result 1: From the low to high concentration group, the inhibitory effect is enhanced.

Possible Result 2: The inhibitory effects of the three different concentration groups differ very little.

3.3. Possible results for treatment duration groups

Possible Result 1: As the processing time becomes longer, the inhibitory effect is positively correlated with the treatment time.

Possible Result 2: Regardless of how long the processing time is, there is no significant relationship between the inhibition effect and the processing time.

4. Discussion

4.1. Possible results for reagent groups

Possible Result 1 supports the hypothesis. The efficient inhibitory effect of SDH1 might contribute to the results, which indicates the potential of SDH1 to cause remission of NSCLC. Furthermore, clinical and pharmaceutical experiments need to be done next to explore the unfounded side effects of SDH1, which is a critical step to put SDH1 into practical application as a new therapeutics.

Possible Results 2 and 5 partially supports the hypothesis. There might be several factors to stop SDH1 functioning, like enzymes with decomposition effect, and substances that can react or combine with it to fail it. As for possible result 2, the obstructive factors prevent SDH1 from functioning its inhibitory effect in vitro, which indicates that SDH1 is only effective in vitro under current conditions. As for possible result 5, SDH1 functions on the molecular level (reduce ALK protein), but the obstructive factors prevent its inhibitory effect at the cellular and tissue level (uncontrolled cell and tumor growth). To confirm the practicability of applying SDH1, further experiments are need to be done to find the obstructive factors and the solution.

Possible Results 3 and 7 partially support the hypothesis. The common explanation is that SDH1 might reduce the volume of NSCLC cells rather than their number to inhibit the aggregate growth of tumor. As for possible result 3, the function of SDH1 is exerted through targeting ALK. As for possible result 7, other effects of SDH1 might help to control the growth of tumor. Because SDH1 has an advantage in reducing tumor volume, there is still research value to confirm the real reason for the shrunk tumor.

Possible Result 4 partially supports the hypothesis. The result indicates that SDH1 has cytotoxicity. It might exert an inhibitory effect to control the tumor growth through functioning on other receptors, rather than targeting ALK specifically. The goal of the next stage experiment is supposed to be finding the effective receptors.

Possible Result 6 partially supports the hypothesis. The only decrease in cell growth indicates the cytotoxicity of SDH1 targeting other receptors. The possible explanation might be that SDH1 could reduce the number of NSCLC cells but their volume grows. To find the effective receptors and the factors that stimulate cell enlargement, more experiments need to be done in the next stage.

Possible Result 8 does not support the hypothesis. There might be two reasons. The first one is that SDH1 is not effective for NSCLC cell and its ALK kinase. Or some factors, as the above discussion, make SDH1 fail to function. The next stage of experiments could focus on finding the contributing factors. Or it would be of great value to use other ingredients to repeat the experimental operation.

4.2. Possible results for concentration groups

Possible Result 1 supports the hypothesis. To combine the best effect and the most economical cost, the next stage of experiments will use mathematical models to delve into the relationship between different concentrations of drugs and inhibitory effects.

Possible Result 2 does not support the hypothesis. The next experimental step will focus on finding the lowest concentration (the most economical cost) with an inhibitory effect.

4.3. Possible results for treatment duration groups

Possible Result 1 supports the hypothesis. Further experiment on treatment time can combine concentration changes, which takes drug resistance into account. It might facilitate clinical and pharmaceutical applications.

Possible Result 2 does not support the hypothesis. Experiments on drug resistance and long-term side effects should be done.

5. Conclusion

In conclusion, this study explores the effect of SDH1 on anaplastic lymphoma kinase (ALK) and A549 NSCLC. The possible results of the study might indicate whether targeting anaplastic lymphoma kinase (ALK) specifically and restrain its activity in vitro and in vivo could cause remission of non-small cell lung cancer. The effect might be able to provide a available way for future research to against NSCLC both clinically and pharmaceutically.

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