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Does oridonin inhibit the growth of small-cell lung cancer?

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Abstract. Research Question: Can oridonin inhibit small cell lung cancer growth by blocking the Notch signalling? Purpose: Small cell lung cancer (SCLC) is an aggressive illness with a low 5-year survival rate. Oridonin is a Chinese medicine extracted from the leaves of the Rabdosia rubescens, a traditional Chinese medicinal herb, which has been proven to have many medical effects in opposition to the tumour. Notch signalling is an essential pathway in multicellular organisms and transfers information into living organisms. Methods: This study will use a human small-cell lung cancer cell line (H1688). Migration assay will test the influence of oridonin on cell migration. A Xenograft mouse tumour model is created to determine the effect of oridonin on tumour growth. Annexin V will test cell apoptosis, and a western blot is used to test whether Notch signalling is activated. All the assays are repeated three times, and the statistics are analyzed by calculating the means and doing the student's t-test. Possible results: There are three main possible results:(1) Oridonin can inhibit SCLC growth by blocking the notch signalling. (2) Oridonin can inhibit the tumour growth of SCLC cells but not by blocking notch signalling. (3) Oridonin cannot inhibit tumour growth but can block the notch signalling. Conclusion: The study will show whether oridonin can inhibit the growth of SCLC by blocking the notch signalling. It will provide a new method of treatment for those with SCLCs.

Keywords: notch signalling, oridonin, SCLC.

1. Introduction

Cancer may be a malady incorporating cells within the body that develop beyond our control. Lung cancer is named as it first takes place in the lungs. Lung cancer may transfer to other more distant absent organs in the human body; take the brain, for example. Lung cancers are usually grouped into two main types: small and non-small cells (including adenocarcinoma and squamous cell carcinoma). These sorts of lung cancer develop unexpectedly and are dealt with unexpectedly. Small cell lung cancer is less common than non-small lung cancer [1]. It is smoking that by distant the driving chance figure for lung cancer. Up to or more than 80% of lung cancer deaths are found to be the consequence of smoking, and this number is likely indeed higher for small-cell lung cancer (SCLC)[2]. People with SCLC may encounter the following symptoms or signs: Fatigue, Cough, Shortness of breath, and Chest pain. According to a previous study, lung cancer has the highest cancer death rate among various cancer diseases. From 1983 to 2012, the survival rate of SCLC patients has kept steady. Five-year survival rates have expanded from 4.9% (1983 through 1993) to 6.4% (2002 through 2012), while the median survival has remained stable at seven months. Relative survival rates (RSRs) have kept stable as well. Be that as it may, more significant survival advancements have occurred in more youthful patient groups [3]. The 5-year survival rate of SCLC is <5% due to the late diagnosis of most SCLC patients [4]

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Oridonin is a natural hetero-pentacyclic compound and ent-karate diterpenoid with the molecular formulae $C_{20}H_{28}O_6$. Oridonin is confined from the leaves of the therapeutic herb Rabdosia rubescens [5]. It includes a part as an antineoplastic agent, an angiogenesis inhibitor, an anti-asthmatic agent, an apoptosis inducer, a plant metabolite, and an antibacterial agent [6].

Notch signalling could be a preserved pathway through advancement in multicellular life forms that directs cell-fate assurance aimed at improvement and keeps up adult tissue homeostasis [7]. The Notch pathway regulates cell expansion, cell density, differentiation, and cell death in all organisms. The notch itself could be a cell-surface receptor which can transduce short-range signals by connecting with transmembrane ligands such as Delta (termed Delta-like in humans) on neighbouring cells [8]. Inside the tumour microenvironment, Jagged ligands can be actuated by tumour-associated growth variables such as VEGF[9] followed by activating Notch expressed in tumour endothelial cells [10]. Moreover, Notch activity was mainly upregulated within the tumour endothelium, proposing that interfering with Notch action may unfavourably influence tumour neo-angiogenesis. A few Notch inhibitors, such as RO4929097 [11] and MK-0752 [12], have, as of now, already been utilized in clinical trials. Hence, focusing on the Notch pathway in endothelial cells might give a valuable strategy for antiangiogenic therapies [13].

Hypothesis: I predict that treatment with increasing concentrations and for various durations with Oridonin can inhibit the growth and migration of H1688 small cell lung cancer cells by blocking Notch cleavage leading to less notch signalling.

2. Materials and methods

2.1. Chemical, reagents and animals

Oridonin, Matrigel, Annexin V/PI and Notch inhibitor DAPT were obtained.

Sprague Dawley (SD) rats and nude mice were purchased.

2.2. Cell lines and cell culture

A human small cell lung cancer cell line (H1688) was cultured at 37°C in a humidified atmosphere containing 5% CO2.

2.3. Migration assay

In brief, cell lines were placed on Matrigel with or without a gradient of Oridonin concentrations with the addition of VEGF (20 ng/ml). An OLYMPUS inverted microscope was used to take photomicrographs after roughly eight hours. Using Image-Pro Plus 6.0 software, tubular structures were accessible, and the inhibition share was previously expressed as 100% when using untreated wells. A modified Boyden chamber assay and a wound healing migration test were used to measure the migration of small cell lung cancer cell lines. Before being incubated with VEGF (20ng/ml) or Oridonin for between 8 and 12 hours, cell lines were pretreated with the positive control notch inhibitor DAPT for two hours. Moving cells have been manually added up and photo-miniaturized to size. It was done using the modified Boyden chamber model (Trans well, 8.0mm pore size; Costar). Cell lines that were 80% confluent underwent 24-hour serum starvation. Another was plating cell lines into the trans well insert using 100cc serum-free media. For the following step, 600 ml of new fundamental ECM medium containing ten ng/ml vascular endothelial growth factor (VEGF) and a slope of concentrations of Oridonin were included in the bottom well. After 4 hours of hatching, cells were washed with PBS to induce freed of the un-invaded cells, fixed with 4% paraformaldehyde taken after by staining with crystal violet. A DXM1200 digital camera and an Olympus IX20 inverted microscope were used to picture and count the migrating cells.

2.4. Xenograft mouse tumor model

H1688 cells suspended in 50 ml PBS were injected subcutaneously into the right flank of male nude mice that were 5–6 weeks old. The mice were randomly assigned to the Oridonin treatment group (n58)

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or the positive control group (DAPT) after the tumour volume reached 150 mm3. An intraperitoneal infusion of oridonin (7.5 mg/kg) was administered daily. The tumour volume was calculated by taking measurements with a digital vernier calliper.

2.5. Annexin V/PI by FACS

SCLC cells should be incubated for 20 minutes at 55 degrees and for the remaining 37 degrees in a humidified environment with 5% CO2. To distinguish between the positive and negative peaks, combine them. Plan groups with various oridonin concentrations and DAPT as a positive control. By combining 5 mL of the 1 mg/mL PI stock solution with 45 mL of 1X Annexin-binding buffer, you can create a working solution of PI at a concentration of 100 g/mL. The leftover portion of this functioning arrangement is saved for upcoming research. After the brooding period, gather the cells and wash them in cold phosphate-buffered saline (PBS). Plan a suitable volume of 100 L per measure and resuspend the cells in 1X Annexin-binding buffer at a concentration of 1106 cells/mL. The taking after step incorporates Annexin V and < 1 μL 100 μg/mL PI working solution to each 100 μL of cell suspension. The cells are brought forth at room temperature for 15 minutes inside the dim. After the hatching period, include 400 μL 1X Annexin-binding buffer, blend them delicately, and keep the tests in a frosty environment. Analyze the stained cells by flow cytometry for as before long as conceivable. The ultimate step is to run the tests on LSR II.

2.6. Western blot assay

Proteins are extracted from cells incubated (standard, positive control DAPT and groups with different concentrations of oridonin). The sample is loaded in the well of SDS-PAGE Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The proteins are separated based on an electric charge, molecular weight, isoelectric point, or a combination. Then place the nitrocellulose membrane on the gel. The separated protein from gel gets transferred to nitrocellulose paper through capillary action. Antibodies, also proteins, are likely to bind the nitrocellulose paper. Subsequently, the membrane is non-specifically immersed or veiled by utilizing casein or Bovine serum albumin (BSA) sometime recently, including the essential counteracting agent. The primary antibody (1° Ab) is specific to protein (Notch receptors), so it will form an Ag-Ab complex. The secondary antibody is enzyme labelled. To image the enzyme activity, the reaction mixture is brooded with a particular substrate. The enzyme will change over the substrate to deliver the visible coloured product, so a band of colour can be seen within the layer.

2.7. Statistical analysis

All the assays are repeated three times, and the statistics are analyzed by calculating the means and doing the student's t-test.

3. Results

3.1. Individual results

3.1.1. Cell migration blocked. Possible result 1: Oridonin can induce cell migration; the migrated cells declined due to the presence of oridonin. When incubated with VEGF and Oridonin, H1688 cells wound healing and trans good migration were suppressed dose-dependent.

Possible result 2: Oridonin has no significant effect on cell migration; the number of migrated cells is unchanged with the addition of oridonin. When incubated with VEGF and Oridonin, H1688 cells wound healing and trans good migration do not present a significant difference.

Possible result 3: Oridonin can promote cell migration; the number of migrated cells increased after adding oridonin.

3.1.2. Tumour growth inhibited. **P**ossible result 6: Oridonin addition can inhibit tumour growth, resulting in smaller tumour volume than in controlled groups.

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Possible result 7: Oridonin has no significant influence on tumour growth; adding oridonin does not inhibit the growth of the tumour.

3.1.3. Cell apoptosis induced. Possible result 8: Oridonin can induce apoptosis of SCLC cells, with fewer SCLC cells surviving.

Possible result 9: Oridonin has no significant effect on the apoptosis of SCLC cells; the number of surviving SCLC cells is not a big difference.

3.1.4. Notch signalling blocked. Possible result 10: Oridonin can block the Notch signalling, reducing the number of notch receptor proteins activated.

Table 1. Possible result 11: Oridonin does not block the notch signalling; the same amount of notch receptor proteins are activated.

CR	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Cell migration blocked by transwell assay?	+	-	+	+	+	-	-	-	+	+	+	-	-	-	+	-
Xenograft Tumor growth inhibited?	+	+	-	+	+	-	+	+	-	-	+	-	-	+	-	-
Cell apoptosis induced by AnnexinV/PI FACS?	+	+	+	-	+	+	-	+	-	+	-	-	+	-	-	-
Does WB block Notch cleavage?	+	+	+	+	-	+	+	-	+	-	-	+	-	-	-	-
Supporting Hypothesis?	YES	P	P	P	P	P	P	P	P	P	P	P	P	P	P	NO

Note. "+" represents the result closer to the positive control (DAPT) "-" represents the result nearer to the negative control (saline). P=Partially.

4. Combination of possible results

CR1: The cell migration is blocked, xenograft tumour growth is inhibited, cell apoptosis is induced, and the notch cleavage is blocked. The result tends to be closer to the positive control as the concentration and the duration of oridonin go higher, which both support the hypothesis: treatment with increasing concentrations and for various durations with Oridonin can inhibit the growth and migration of H1688 small cell lung cancer cells by blocking Notch cleavage leading to less notch signalling. It is an ideal group of results.

The combination results in 2-15 have some of the results nearer to the negative control group (standard). For example, CR2 shows that oridonin can block cell migration, inhibit xenograft mouse tumour growth, and induce cell apoptosis but have no significant effect on blocking the notch signalling. CR3 shows that oridonin cannot inhibit the xenograft tumour growth but can block cell migration, induce apoptosis, and block the Notch cleavage. CR4 shows that oridonin may not be efficient in inducing cell apoptosis but can complete the remaining functions. CR5 shows that oridonin does not block the Notch cleavage but can achieve the other three expectations.CR6-11 have two results closer to the negative control group, showing that oridonin may not be efficient when treating the SCLCs. CR12-15 only provides that oridonin can hardly complete all the tasks of blocking cell migration, inhibiting xenograft tumour growth, inducing cell apoptosis, and blocking the notch cleavage. However, the results may change when using higher concentrations and longer durations of oridonin. CR16: None of the results is closer to the positive control group, which shows that the hypothesis may be wrong. However, it may be caused by small mistakes during the assays, insufficient oridonin, or the shortness of duration.

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5. Discussion

Combination result 1 has all the results nearer to the positive control group (DAPT), which can support the hypothesis. The results are more significant when higher concentrations of oridonin and longer durations are used. It shows that oridonin can inhibit the growth and migration of H1688 small-cell lung cancer cells by blocking Notch cleavage leading to less notch signalling. Further investigation could be measuring the potential side effects and determining the specific mechanism and medical delivery measures.

CR2-15: These results partly support the hypothesis, with not all having all the results closer to the positive control group. CR2 shows that oridonin can block cell migration, inhibit xenograft mouse tumour growth, and induce cell apoptosis but have no significant effect on blocking the notch signalling. CR3 shows that oridonin cannot inhibit the xenograft tumour growth but can block cell migration, induce apoptosis, and block the Notch cleavage. CR4 shows that oridonin may not be efficient in inducing cell apoptosis but can complete the remaining functions. CR5 shows that oridonin does not block the Notch cleavage but can achieve the other three expectations.CR2-5 are close to the positive results: each has only one result close to the positive ones.

CR6-11 have two results closer to the negative control group, showing that oridonin may not be efficient when treating the SCLCs. They show that oridonin may not complete all the missions, but maybe oridonin can make a more significant difference when used with other medications.

CR12-15 only provide that oridonin can hardly complete all the tasks of blocking cell migration, inhibiting xenograft tumour growth, inducing cell apoptosis and blocking the notch cleavage.

This does not mean that oridonin is useless when dealing with SCLC; it may have special effects when facing other cancers like breast cancer. They may be due to insufficient amount of oridonin or duration shortness. They show that the hypothesis is not entirely true but contains some right points.

CR16: None of the results is closer to the positive control group, which shows that the hypothesis may be wrong. However, it may be caused by small mistakes during the assays, insufficient oridonin, or the shortness of duration.

The results show that oridonin may not play its role in inhibiting tumour growth in vitro and in vivo by the specific way in the hypothesis. Future research can investigate oridonin's effect on notch cleavage or varying concentrations and durations to understand the results better.

Combination results in 16 show an opposite result compared to the hypothesis. Using oridonin cannot inhibit cell migration, induce cell apoptosis, inhibit xenograft mouse tumour growth or block the notch cleavage. Small mistakes may cause the results during the assays, insufficient oridonin, or shortness of duration. Alternatively, oridonin does not function in the mouse. The experiment ought to be repeated. Future research can investigate the effect of oridonin on tumour inhibiting in vitro and in vivo.

6. Conclusion

This study shows whether oridonin can inhibit the growth of SCLC by blocking the notch signalling. It will provide a new treatment method to those with SCLC if possible.

References

[1] Zhi-Mei, Zhao, Shi-Chao, et al. Treatment of Skin Reaction Induced by Nivolumab Combined with Radiotherapy in Non-small Cell Lung Cancer: A Case Report.[J]. Chung Kuo I Hsueh Ko Hsueh Tsa Chih, 2018.

[2] CD Murphy, JM Lee, B Drohan, DM Euhus, DB Kopans, & MA Gadd. (2008). The american cancer society guidelines for breast screening with magnetic resonance imaging : an argument for genetic testing. Cancer.

[3] *Min, J. , Lee, H. , Lim, H. , Ahn, M. , Park, K. , & Chung, M. , et al. (2011). Drug-induced interstitial lung disease in tyrosine kinase inhibitor therapy for non-small cell lung cancer: a review on current insight. Cancer chemotherapy and pharmacology, 68(5), 1099-109..*

[4] *Landis, S. H. , Murray, T. , Bolden, S. , & Wingo, P. A. . (2010). Cancer statistics, 1998. Ca A Cancer Journal for Clinicians, 48(1), 6-29.*

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[5] Paolo, M., Agnieszka, K. , & Kutas, G. J. . (2001). Cancer chemotherapy: the role of pharmacological agents in the management of hematological malignancies. University of Toronto Medical Journal, 79(1).
[6] Visscher, & Maurice, B. . (1976). National library of medicine. Jama the Journal of the American Medical Association, 235(6), 600.
[7] Bray, S. J. . (2006). Bray sj. notch signaling: a simple pathway becomes complex. nat rev mol cell biol 7: 678-689. Nature Reviews Molecular Cell Biology, 7(9), 35-42.
[8] Wood, M. S. . (2000). U.s. national library of medicine. Health Care on the Internet, 4(1), 80-81.
[9] Shawber CJ, Kitajewski J. "Notch function in the vasculature: insights from zebrafish, mouse and man.". *Bioessays 26.* 2004, pp. 225-234.
[10] JFolkman. “Role of angiogenesis in tumor growth and metastasis.”. .*Semin Oncol 29.* 2002, pp. 15-18.
[11] Tolcher AWWA, Mikulski SM, Papadopoulos KP, Kwak EL, et al.Messersmith. (2012): 2348–23. Phase I study of RO4929097, a gamma secretase inhibitor of Notch signaling, in patients with refractory metastatic or locally advanced solid tumors.”. *J Clin Oncol 30.* 2012, pp. 2348-2360.
[12] Krop IT, Guthrie T, Wen PY, Mason WP, et al.Demuth. “ Phase I pharmacologic and pharmacodynamic study of the gamma secretase (Notch) inhibitor MK-0752 in adult patients with advanced solid tumors.”. . *J Clin Oncol 30.* 2012, pp. 2307-2313.
[13] Kiec-Wilk BJ, Polus A, Pryjma J, Knapp A, et al.Grzybowska-Galuszka. “ The MAPKdependent regulation of the Jagged/Notch gene expression by VEGF, bFGF or PPAR gamma mediated angiogenesis in HUVEC.”. *J Physiol Pharmacol 61.* 2010, pp. 217-225.