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Investigate the role of oridonin in esophageal cancer and candida albicans

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Abstract:

This paper focus on the esophageal cancer, in China, 150000 people died from the esophageal cancer every year, it also has high mortality rate in the world. This report will investigate whether traditional Chinese medicine oridonin (ORI) has an impact on esophageal cancer. Assuming that VX2 (cell line) tumor size and Candida Albicans (CA) are reduced by ORI, ORI will decrease the levels of TNF-α and IL1. We will conduct experiments on rabbits to determine the quantity of CA and TNF-α and IL1 production cytokines through EILSA and endoscopic experiments, and investigate whether these cytokines can cause CA or VX2 cancer. This study will contribute to the development and connection of ORI treat esophageal cancer.

Keywords: Oridonin, VX2 cell line, candida albicans, IL1, TNF-α

1. Introduction

In recent years, cancer is the deadliest in the world. 2.3 million people died from cancer in China in 2019; the proportion of esophageal cancer is 6.26%. Cancer is caused by uncontrolled cell division that it can happen to any part of the body. There are two main types of esophageal cancer: Esophageal squamous cell carcinoma (OSCC) and Adenocarcinoma (OAC). Cancer is a very complex health issue that scientists from various fields around the world study every year. Although the country allocated 220 billion RMB for cancer prevention in 2019, 4.4 million people still had cancer in 2019, and esophageal cancer is one of them. There are currently many methods to treat and prevent cancer, for example, surgery IVOR-LEWIS [1] to remove tumors or use Alpha, beta, and gamma rays produced by radioactive isotopes to irradiate cancer cells, targeted therapy that uses molecular biology differences between tumor cells and normal cells, targeting cell receptors, key genes, and regulatory molecules, designing corresponding therapeutic drugs to intervene in signaling pathways closely related to tumor occurrence, thereby specifically inhibiting tumor growth and metastasis [2]. Radiation therapy can harm a person's healthy cells. This paper is about whether ORI in traditional Chinese medicine can be used to treat esophageal cancer. The ORI is from ardisia rubescent, and It can selectively attack the target antigen, thus not harming other cells and being targeted. If it can treat esophageal cancer, it will bring hope to many patients.

CA, candida albicans is a common fungal pathogen that can cause common mucosal discomfort or life-threatening systemic diseases. Its main cellular pathways include cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) [3], and Candida albicans often cause infections. Figure 1 is shows the morphology of Candida albicans in a laboratory

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culture dish [4].

Figure 1. Morphology of Candida albicans in a laboratory culture dish [4]

Oridonin(Figure 2) is a natural substance with biological activity isolated from Rabdosia rubescens traditional Chinese medicine [5]; it can be isolated from Ardisia rubescent by the HPLC method. It has various biological activities and has certain application value in cancer and inflammation. It can reduce the amount of TNF-α [6]. It can treat inflammation caused by CA that is caused by IL1and TNF-α cytokines [5]. ORI may affect IL1 and TNF-α cytokines.

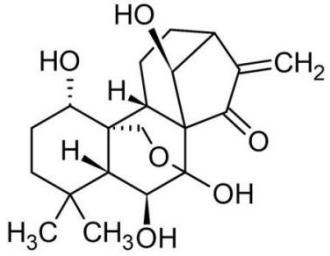

Figure 2. The structure of oridonin

2. IL-1 & TNF-α

IL-1 is one of the important inflammatory mediators and a thermogenic component that has the ability to induce heat and mediate inflammation [7]. It mainly plays a regulatory role in cellular immune activation.IL-1 signal pathway include IL-1 cytokine family that consists of 11 members and plays an important role in inflammation regulation, and plays an important role with TNF-α in the pathogenesis of acute and chronic inflammation. TNF-α is mainly produced by activated macrophages and monocytes, and plays a key role in physiological and pathological processes such as immune regulation, inflammatory response, and cell apoptosis [8].

In this paper, through inject VX2 in rabbit animal model, using high concentration of ORI for long term treatment, determine blood sample from VX2 xenograft rabbits whether ORI effect on number of TNF-α and IL1, and also effect on size of VX2 esophageal cancer tumor size. This study will confirm if there is a connection between ORI and VX2 esophageal cancer.

3. Methods

3.1 VX2 cancer cell culture and ORI treatment

This experiment uses fire rabbits to make compare. Thaw frozen cells in water at 37 degrees Celsius, injected VX2 cell subcutaneously into the thigh for fire rabbits. Measuring the initial tumor size using a digital caliper [9]. Feeding fire rabbits normally but ORI supplements as medicine to treat four of the rabbits in different concentration, lasting for 8 weeks. Experiments can be conducted using different concentrations of ORI, and several dilution methods can be used to divide the concentrations into (100, 10, 1, 0.1, 0.01) uM. Duration: 6h,12h,18h,24h lasting for 8weeks. Rabbits treated with different concentration divide into different groups such as class 1,2,3,4 [10].

3.2 Measure the IL1 and TNF-α expression level by sandwich ELISA essay

Add primary antibodies to microplates to capture IL1 molecules, then add rabbit serum to the microplate, next add secondary antibodies to bind IL1 molecules with complementary shape, and add enzyme to react with secondary antibodies, forming a color product. Microplate Reader is used to detect IL1 quantity in rabbit serum [11]. At the beginning and end of medication, use ELISA method to detect once each, and then compare with positive control and negative control. Positive control: 5-Fluorouracil (5FU), negative control DMSO/PBS

3.3 Measure the size of VX2 cancer tumor size

Detect the tumor size of four rabbits before the start of treatment, cut the mature tumor from each rabbit after eight weeks of treatment, record the data of each class rabbits. Elliptical sphere calculation method is used in the experiment to measure the VX2 cancer tumor size. Formula is $\pi/6\times L\times W\times H$, then use the difference in tumor between fire rabbits is calculated to determine whether the drug has reduced the tumor size in the rabbits.

3.4 Endoscopic examination of Candida albicans in the esophagus

Before administering medication to rabbits, we placed the rabbit on a tilted table in a supine position at approximately 10 degrees, inject water into the esophagus for lubrication, then insert a 3.8mm PAE-EUS probe forward

[12]. Find the location of the tumor and observe if there are white oval shaped CA attached to the esophagus in the attachment, using endoscopic photos to estimate the number of CA, and after eight weeks of medication, use an endoscope to take photos and record them, afterwards, compare the difference in the number of CA between the two sets of photos.

3.5 Statistical methods

The experiment will be repeated for three times, in each experiment. The data collected from fifteen rabbits (rabbits treated with ORI and rabbits not treated with ORI) will be used for comparison. T-test will be use to compare the significance of result with the control groups for ELISA assay. A significance level of $p<0.05$ will be considered in this experiment to indicate the statistical significance.

4. Result

Table 1 possible results

Combination of possible results (CR)	Oridonin Decreases candida albicans by endoscopy?	Oridonin decreases VX2 tumor size in xenografts?	Oridonin decreases TNF-α by ELISA?	Oridonin decreases IL1 by ELISA?	Support of hypothesis
CR1	+	+	+	+	Yes
CR2	+	+	+	-	Partially
CR3	+	+	-	+	Partially
CR4	+	-	+	+	Partially
CR5	-	+	+	+	Partially
CR6	+	+	-	-	Partially
CR7	+	-	+	-	Partially
CR8	+	-	-	+	Partially
CR9	-	+	-	+	Partially
CR10	-	-	+	+	Partially
CR11	-	+	+	-	Partially
CR12	+	-	-	-	Partially
CR13	-	+	-	-	Partially
CR14	-	-	+	-	Partially
CR15	-	-	-	+	Partially
CR16	-	-	-	-	No

Table legend: "+" indicates Observed phenomena in each column title, the result is the same as the positive control in ELISA essay. "-" indicates Not observing every phenomenon in each column, the result is the same as the negative control in ELISA essay.

4.1 Description of each combination

Combination of possible result 1 (CR1)
Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell also decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α both decrease.
Combination of possible result 2 (CR2)
Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell also decrease. Comparing with positive and negative controls, the level of TNF-α decrease but the levels of IL1 has not decreasing.
Combination of possible result 3 (CR3)
Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell also decrease. Comparing with positive and negative controls, the levels of IL1 decrease but the levels of TNF-α has not decreasing.
Combination of possible result 4 (CR4)
Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell doesn't decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α both decrease.
Combination of possible result 5 (CR5)

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Comparing with photos in esophageal before the treatment, the number of CA has not decrease during this experiment. The size of VX2 cancer tumor cell decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α both decrease.

Combination of possible result 6 (CR6)

Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell also decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α both show no decrease.

Combination of possible result 7 (CR7)

Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell not decrease. Comparing with positive and negative controls, the levels of TNF-α decrease but the levels of IL1 not decreasing.

Combination of possible result 8 (CR8)

Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell not decrease. Comparing with positive and negative controls, the levels of IL1 decrease but the levels of TNF-α has not decreasing trend.

Combination of possible result 9 (CR9)

Comparing with photos in esophageal before the treatment, the number of CA not decrease. The size of VX2 cancer tumor cell decrease. Comparing with positive and negative controls, the levels of IL1 decrease but the levels of TNF-α not decreasing.

Combination of possible result10 (CR10)

Comparing with photos in esophageal before the treatment, the number of CA not decrease. The size of VX2 cancer tumor cell also not decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α both decrease.

Combination of possible result 11 (CR11)

Comparing with photos in esophageal before the treatment, the number of CA not decrease. The size of VX2 cancer tumor cell decrease. Comparing with positive and negative controls, the levels of TNF-α decrease but the number of IL1 not decline.

Combination of possible result 12 (CR12)

Comparing with photos in esophageal before the treatment, the number of CA decrease. The size of VX2 cancer tumor cell not decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α also not decrease.

Combination of possible result 13 (CR13)

Comparing with photos in esophageal before the treatment, the number of CA not decrease. The size of VX2 cancer tumor cell decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α both not decrease.

Combination of possible result 14 (CR14)

Comparing with photos in esophageal before the treatment, the number of CA not decrease. The size of VX2 cancer tumor cell also not decrease. Comparing with positive and negative controls, the levels of TNF-α decrease, however the number of IL1 not show decrease.

Combination of possible result 15 (CR15)

Comparing with photos in esophageal before the treatment, the number of CA not decrease. The size of VX2 cancer tumor cell also not decrease. Comparing with positive and negative controls, the levels of IL1 decrease but the number of TNF-α not show decreasing.

Combination of possible result 16 (CR16)

Comparing with photos in esophageal before the treatment, the number of CA not decrease, and other three experiments also show the negative result. The size of VX2 cancer tumor cell not decrease. Comparing with positive and negative controls, the levels of IL1 and TNF-α also not decrease.

5. Discussion

The CR1 results indicate that ORI has a certain inhibitory effect on the growth of CA. The elliptical calculation method also shows that ORI can reduce the size of VX2 cancer tumors. The sandwich ELISA method also shows that ORI has a certain inhibitory effect on IL1 and TNF-α, which is consistent with our hypothesis.

CR2 and CR3 are very similar. In the sandwich ELISA experiment, the binding of antibodies and secondary antibodies did not decrease, which may be due to the gene of IL1 or TNF-α has undergone changes due to natural selection, changing the sequence of amino acid chain, producing more drug-resistant cells, these cells are immune to the effects of ORI. Indicating that ORI can still be used to combat cancer caused by VX2 and inflammation caused by CA, most of which are consistent with the hypothesis. The future experiment uses other medicine such as tripterygium wilfordii to test whether the number of IL1 or TNF-α decreasing.

The CR4 sandwich ELISA experiments all showed positive results, IL1 and TNF-α did not exhibit drug resistance within eight weeks. At the same time, ORI can effectively inhibit the growth of CA, but in terms of the size of VX2 cancer tumor cells, ORI did not reduce the size of tumors. This may be because IL1 and TNF-α are not cytokines that cause VX2 cancer and the reduction of CA will not affect the size of VX2 tumor cells. Regardless, CR4 still supports three hypotheses. The future experiment will detect what cytokines cause VX2 cancer, using the method of cell culture that puts VX2 cancer into different cyto-

kines and see the survival, multiplication, and apoptosis.
CR5 shows that this may be due to CA adapting to ORI, causing ORI to have no effect on CA, or it may be due to CA changing its position and more CA coming to the initial photography location. However, ORI still inhibits VX2 cancer tumor size and IL1 and TNF-α. Most experiments have confirmed the hypothesis, with only one not.
From CR6, ORI had no effect on TNF-α and IL1 cytokines. This is likely because ORI inhibited other cytokines in the VX2 cell line pathway, leading to a decrease in tumor size. The two experimental results correspond to the hypothesis.
In CR7 result. Suggests that it is very likely that IL1 caused VX2 cancer tumor cells, as neither of them was reduced. CA is likely caused by TNF-α, and when TNF-α is reduced, CA will also be reduced. We can conclude that ORI can reduce TNF-α and the CA caused by it. Corresponding to two of our assumptions.
CR8 is a similar inference to CR7. TNF-α is highly likely to be one of the factors causing VX2 tumor cancer. However, it cannot be ruled out that TNF-α affects other areas. Similarly, CA may also be caused by IL1 because both of them decreased throughout the entire experiment. ORI can inhibit CA but has no effect on VX2 cancer tumor cells, two experiments correspond to the hypothesis.
The result of CR9 suggests that CA in the esophagus of rabbits is likely caused by TNF-α cytokines, as both CA and TNF-α were not affected by ORI. However, I believe that ORI can limit IL1 cytokines and reduce VX2 tumor cell size. We can conduct another experiment to verify that TNF-α is a cytokine that causes CA. Using cell culture assays, CA can be cultured in dishes with and without TNF-α to determine if they interact with each other. This experiment can also be used to further detect in CR7, CR10and CR11. CR9 validated two predictions.
Through CR10, we can conclude that it is highly likely that TNF-α and IL1 cytokines are not the main factors causing CA and VX2 tumor cells. Therefore, ORI can reduce the number of TNF-α and IL1, but cannot affect the size of VX2 tumors and the number of CA. We can further conduct cell culture experiments to determine their relationship. Anyway, this result proves that ORI can still reduce the number of TNF-α and IL1, providing two positive results for our hypothesis.
We can infer CR11 using the inference method of CR8, 7, and 9. I believe that IL1 is likely the cytokine that causes CA, as both IL1 and CA have not decreased. TNF-α is also likely to be the factor that causes VX2 cancer cells. ORI reduces TNF-α, so the size of VX2 tumor cells also decreases accordingly. The results indicate that ORI can reduce TNF-α cytokines and VX2 tumor cell size, which is consistent with our two hypotheses.
CR12 demonstrated that highly likely TNF-α and IL1 are not the cytokines causing CA. ORI reduces other cytokines that affect CA, such as the AIs cell family. We can detect whether ORI reduces the number of AIs by using sugar methods and fluorescence immunoassay to allow antibodies and proteins to bind and react with enzymes to produce fluorescence. This result demonstrates that ORI has no effect on the size of VX2 cancer cells, possibly because VX2 is triggered by TNF-α and IL1. This result only corresponds to one of our predictions and does not match our predictions.
CR13 is similar to CR12 because ORI only reduces the size of VX2 cancer tumor cells and does not reduce the number of TNF-α and IL1, so they are not factors that cause VX2 cancer. I think ORI may inhibit other factors, leading to the reduction ratio of VX2 tumor cells, such as p53. ORI may make p53 protein active. We can do gel electrophoresis experiments to verify the activity of p53, And CA is caused by TNF-α and IL1, which only corresponds to one prediction.
The results of CR14 and CR15 show some similarity, as they both only demonstrate the effect of ORI on one of TNF-α and IL1. This also indicates that TNF-α and IL1 are not the main factors causing VX2 tumor size and CA, and their pathways may not affect the size of VX2 cancer tumors and the number of CA. This only shows a positive experimental result in four experiments, so this result is partially supporting hypothesis.
The CR16 experiment is likely to indicate that ORI may have some drug toxicity to rabbits, or it may be due to the fact that the area where ORI is most absorbed in the rabbit's body is not in the esophageal appendage, which cannot affect the size of VX2 tumors and Candida albicans. This result does not support our hypothesis.

6. Conclusion

The results demonstrated whether oridonin has an impact on cancer, strengthen the connection between traditional Chinese medicine and cancer, add a branch to the field of anti-cancer, and explore whether ORI affect other cancers such as lung cancer and liver cancer in subsequent experiments.

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