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Andrographolide stimulates senescence in colon cancer through p21 and p27

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Abstract. Colon cancer is a common malignant tumor of the digestive tract that occurs in the colon, which belongs to non-infectious diseases, and is collectively referred to as colorectal cancer with rectal cancer. Globally, approximately 1.09 million new cases of colorectal cancer are diagnosed annually, resulting in 550,000 deaths. The incidence of colorectal cancer is projected to increase by 60% globally by 2030.Recent data indicate that colon cancer patients account for up to 70% of colorectal cancer patients, despite surgery and adjuvant therapy, 5%-30% of colon cancer patients have recurrence, thus colon cancer remains a major global health problem. Andrographolide is a diterpene lactone isolated from the Traditional Chinese Medicine Andrographis paniculate with anti-inflammatory, hepatoprotective and anti-infective effects. Some studies have shown that it can provide help in the treatment of breast cancers by affecting p21 and p27. We investigated the anti-colon cancer mechanism of Andrographolide by studying the effect of Andrographolide on p21 and p27 of colon cancer HCT116 cells, expecting to provide theoretical support for the treatment of colon cancer by investigating whether andrographolide can induce senescence of human colon cancer HCT116 cells by causing an increase in p21 and p27, thereby reducing the survival rate of HCT116.

Keywords: Andrographolide, colon cancer, cell senescence, p21, p27.

1. Introduction

Colon cancer is one of the world's leading tumors and is regarded as the biggest killer, along with lung, prostate as well as breast cancer.[1-3]. Despite great efforts have been made, colon cancer remains a recalcitrant disease. It is predicted that in 2020, global number of new cases of colorectal cancer will be 19,315,590, with a world standard rate of 19.5 per 100,000, and the number of deaths due to colorectal cancer will be 935,173, with a world standard rate of 9.0 per 100,000, which makes the incidence rate and mortality rate of colorectal cancer ranked the fourth and the third among the world's major malignant tumors, respectively. The incidence and mortality rates of colorectal cancer in China are 17.3 per 100,000 and 7.8 per 100,000 respectively[4].While most patients with colon cancer are diagnosed at an early stage, cancer frequently recurs after initial eradicative treatment, highlighting the imperative demand for new methods. The challenges in the current treatment paradigm are various, including the lack of accurate patient selection tools for complementary therapies, toxicity of disability, as well as modest efficacy of the adjunctive therapies[5].

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Andrographolide is a diterpene lactone isolated from the medicinal plant Andrographis paniculata with anti-inflammatory, hepatoprotective and anti-infective effects. In recent years, the anticancer effects of Andrographolide have attracted extensive attention from researchers.

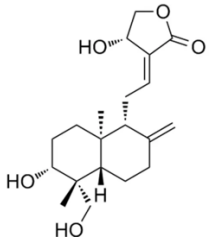

Figure 1. Andrographolide

The P21 gene is a significant member of the family of cell cycle protein-dependent kinase inhibitors discovered in the past few years. It is tightly linked to tumor inhibition and can mediate the interrelationships between the cell cycle, DNA replication and repair by suppressing the activity of the cell cycle protein-dependent kinases CDKs, thus connecting tumor suppression to cell cycle control. p21 gene discovery, cloning and its role in cell cycle regulation and tumorigenesis have important roles. The discovery, cloning, and role of the p21 gene in cell cycle regulation and tumorigenesis are important. P21 gene polymorphisms have been associated with susceptibility to some malignant tumors.

p27 is a cell cycle protein-dependent kinase inhibitor (CDKI). It is capable of blocking the transition from G1 to S phase of the cell cycle by binding to or inhibiting cyclin or cyclin-dependent kinase (CDK). Studies have shown that the expression level of p27 is important for the prognosis of malignant tumors such as breast cancer, prostate cancer, gastrointestinal cancer, lung cancer, etc., and low p27 expression indicates that patients have a poorer prognosis.

Senescence is a stable cell cycle arrest which can be activated by carcinogenic signals and manifested by changes in cellular organization as well as gene expression, for instance, the induction of a complex secretome. What needs to be emphasized is that senescence restricts tumor progression and plays a rather crucial role in the efficacy of conventional anticancer therapies. For the past few years, therapies such as p53 reactivation, inhibition of c-MYC in addicted tumors or treatment with cyclin-dependent kinase (CDK) inhibitors have proven effective by invoking a senescence response. The possibility of utilizing senescence therapies for cancer treatment has attracted considerable interest. It has been proposed that the senescence secretome could be a source of new targets for pre-senescence therapies because of its tumour-suppressive effects. In short, customized pre-senescence therapies hold promise for treating cancer and other diseases[6].

Research indicated that Andrographolide (AD) stimulated lung adenocarcinoma senescence in vitro and in vivo via p53/p21 and Skp2/p27. Thus, AD is a promising senescence-inducing therapy used for the treatment of stubborn human cancers[7].Meanwhile, other research pointed out that p21 and p27 in colon cancer cells also play an important role in cellular senescence. However, limited researches are focused on the relationship between p21, p27and colon cancer. I expect to study the effect of Andrographolide on p21, p27 of colon cancer HCT116 cells to provide theoretical support for the treatment of colon cancer cells.

Hypothesis:

I predict that Andrographolide is able to induce senescence in human colon cancer HCT116 cells through an increase in p21 and p27 resulting in decreased HCT116 viability.

2. Material and Methods

In this experient, Positive control is MLN4924 and negative control is 0.1%DMSO.

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2.1. Cell media and reagents

Human colon cancer cells (HCT-116) were routinely cultured in medium. Andrographolide was dissolved in DMSO and kept at −20◦C before use. The purity of andrographolide is 98%.

2.2. Antibodies

Antibodies against p21, p27 were diluted at 1:1000.

2.3. Cell viability assay

Cell viability by MTT assay. Cell culture: HCT-116 were cultured in RPMI-1640 medium containing 10% fetal bovine serum, and incubated in a constant temperature incubator at 37°C with 5% CO2 until logarithmic growth phase. MTT reagent treatment: Cells were inoculated into 96-well plates with about 1×10^4 cells per well, and cultured overnight, then 98% Andrographolide (experimental group), 0.1%DMSO (negative control) and MLN4924 (positive control) were added, respectively, and 4 replicate wells were set up for each group; The experimental group was subdivided into four groups according to the incubation time, and the incubation time of each group was 12 hours, 24 hours, 36 hours and 48 hours, respectively. Negative and positive control groups were also grouped in the same way as described above. MTT assay: after reaching the treatment time of each group, 10 μL of MTT solution (5 mg/mL) was added to each well, and the incubation was continued for 4 hours. Discard the medium and add 150 μL of 0.1%DMSO to dissolve the purple crystals formed, and gently shake for 10 minutes to fully dissolve the crystals. Absorbance measurement: The absorbance (OD value) of each well was measured at 570 nm utilizing an enzyme marker to calculate the cell viability.

2.4. Western blot

The abundance of p21 and p27 by western blot. Material: Human colon cancer cells HCT-116, Andrographolide, MLN4924 (positive control),0.1%DMSO (negative control), RIPA lysis buffer, Protein Quantification Kit, Western Blot related reagents and equipment, Anti-p21, anti-p27 and anti-β-actin (internal reference) antibodies. Methods: Cell culture: HCT-116 cells were cultured to logarithmic growth phase. Treatment group setting: three groups of cells were set up with 98% Andrographolide (experimental group), 0.1%DMSO (negative control) and MLN4924 (positive control). The experimental groups were divided into four groups according to the incubation time, and the incubation time of each group was 12 hours, 24 hours, 36 hours and 48 hours, respectively. Negative control and positive control groups were similarly grouped as described above. Protein extraction: Total cellular protein was extracted using RIPA lysis buffer according to the manufacturer's instructions. Protein quantification: The protein concentration of each sample was determined using a protein quantification kit. Western Blot: Standard Western Blot procedures were followed, including SDS-PAGE electrophoresis, protein transfer, antibody incubation and signal detection. Data analysis: The expression levels of p21 and p27 were quantified utilizing image analysis software as well as normalised to the internal reference β-actin.

2.5. Measure senescence

Cell culture: HCT-116 cells were cultured to logarithmic growth phase and grouped according to the experimental design. Treatment conditions: 1. Experimental group: add 98% Andrographolide to treat cells.2.Negative control group: add equal volume of 0.1%DMSO to treat cells.3.Positive control group: cells were treated with the addition of MLN4924, a compound known to induce cellular senescence. Staining assay: Staining was performed according to the instructions of the β-galactosidase staining kit. Microscopic observation: observe the stained cells using a light microscope and record the number and morphological changes of senescent cells (blue granules). Data analysis: the percentage of senescent cells in each group was calculated and statistically analysed to assess the effect of Andrographolide on the senescence of HCT-116 cells.

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2.6. Student T test

Student T test will be used to analyze the data produced by MTT assay.

3. Results

3.1. Possible Results Table

i.e.

Table 1. Combinations of possible results from different experiments

Combination Result # (CR#)					
	Andrographolide increases senescence by Bgal assay	Andrographolide increases p21 by WesternBlot	Andrographolide increases p27 by WesternBlot	Andrographolide decreases viability by MTT	Support of hypothesis
1	+	+	+	+	Full
2	+	+	+	-	Partial
3	+	+	-	+	Partial
4	+	-	+	+	Partial
5	-	+	+	+	Partial
6	+	+	-	-	Partial
7	+	-	-	+	Partial
8	-	-	+	+	Partial
9	+	-	+	-	Partial
10	-	+	-	+	Partial
11	-	+	+	-	Partial
12	+	-	-	-	Partial
13	-	+	-	-	Partial
14	-	-	+	-	Partial
15	-	-	-	+	Partial
16	-	-	-	-	Fully Contradicts

Table legend: "+" represents a positive result which is consistent with the positive control. "-" represents a negative result which is cosistent with the negative control group.

CR1: If I obtain this result I would see increasing senescence in HCT116 occurs, p21increases, p27 increases, the viability of HCT116 decreases. This result Fully supports hypothesis.

CR2: If I obtain this result I would see increasing senescence in HCT116 occurs, p21increases, p27 increases, the viability of HCT116 doesn't change. This result partially supports hypothesis.

CR3: If I obtain this result I would see increasing senescence in HCT116 occurs, p21 increases, p27 doesn't increases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR4: If I obtain this result I would see increasing senescence in HCT116 occurs, p21 doesn't increase, p27 increases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR5: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 increases, p27 increases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR6: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 increases, p27 doesn't increases, the viability of HCT116 doesn't change. This result partially supports hypothesis.

CR7: If I obtain this result I would see increasing senescence in HCT116 occurs, p21doesn't increase, p27 doesn't increase, the viability of HCT116 decreases. This result partially supports hypothesis.

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CR8: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 doesn't increase, p27 increases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR9: If I obtain this result I would see increasing senescence in HCT116 occurs, p21 doesn't increase, p27 increases, the viability of HCT116 doesn't change. This result partially supports hypothesis.

CR10: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 increases, p27 decreases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR11: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 increases, p27 increases, the viability of HCT116 doesn't change. This result partially supports hypothesis.

CR12: If I obtain this result I would see increasing senescence in HCT116 occurs, p21decreases, p27 increases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR13: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 increases, p27 decreases, the viability of HCT116 doesn't change. This result partially supports hypothesis.

CR14: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 decreases, p27 increases, the viability of HCT116 doesn't change. This result partially supports hypothesis.

CR15: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 decreases, p27 decreases, the viability of HCT116 decreases. This result partially supports hypothesis.

CR16: If I obtain this result I would see increasing senescence in HCT116 doesn't occur, p21 decreases, p27 decreases, the viability of HCT116 doesn't change. This result fully contradicts hypothesis.

4. Discussion

CR1: I think, about this result, I would see possible result 1 fully support my hypothesis. Under Andrographolide treatment, the expressions of p21 and p27 are increased, therefore increasing senescence in HCT116 occurs, resulting in decreased HCT116 viability.

CR2: I think, about this result, I would see possible result 2 partially support my hypothesis. Under Andrographolide treatment, the expressions of p21 and p27 are increased, therefore increasing senescence in HCT116 occurs, however, the viability of cells doesn’t decrease significantly. The absence of significant changes in cell viability may be due to the insufficient duration of treatment with andrographolide.

CR3: I think, about this result, I would see possible result 3 partially support my hypothesis. Under Andrographolide treatment, the expressions of p21 is increased, therefore increasing senescence in HCT116 occurs, resulting in decreased HCT116 viability. The absence of increase in P27 may indicate that andrographolide did not have a significant effect on the expression of P27 and that P27 doesn’t play an important role in the regulation of cellular senescence. Andrographolide may regulate cellular senescence in other ways, such as P16 or P53 which are also significant components in cell cycle regulation.

CR4: I think, about this result, I would see possible result 4 partially support my hypothesis. Under Andrographolide treatment, the expressions of p27 is increased, therefore increasing senescence in HCT116 occurs, resulting in decreased HCT116 viability. The absence of increase in P21 may indicate that andrographolide did not have a significant effect on the expression of P21 and that P21 doesn’t play an important role in the regulation of cellular senescence. Andrographolide may regulate cellular senescence in other ways, such as P16 or P53.

CR5: I think, about this result, I would see possible result 5 partially support my hypothesis. Under Andrographolide treatment, the expressions of p21 and p27 are increased, therefore decreasing viability in HCT116 occurs, however, the senescence of cells doesn’t occur. This suggests that cells may not undergo a decline in viability through cellular senescence, and that andrographolide may directly stimulate or inhibit cellular activity and affect normal cellular function.

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CR6: I think, about this result, I would see possible result 6 partially support my hypothesis. Under Andrographolide treatment, p21 increases, leading to senescence in cells occurs. The absence of significant changes in cell viability may be due to the insufficient duration of treatment with andrographolide.Meanwhile,P27 doesn't play an important role in the regulation of cellular senescence.

CR7: I think, about this result, I would see possible result 7 partially support my hypothesis. Instead of by p21 and p27,Andrographolide induces senescence in other way, for instance,TLR4/NF-κB/MMP-9 signaling pathway, which suppresses proliferation of human colon cancer.

CR8: I think, about this result, I would see possible result 8 partially support my hypothesis. Under Andrographolide treatment, p27 increases, leading to decreasing viability in cells occurs. This result may be due to an error in the experimental procedure and the experiment should be repeated several times to further ensure the accuracy of the results.

CR9: I think, about this result, I would see possible result 9 partially support my hypothesis. Increasing senescence in cells is due to increasing p27,while p21 might have little effect in cell senescence. The absence of increase in P21 may indicate that andrographolide did not have a striking effect on the expression of P21.The absence of striking changes in cell viability may be due to the insufficient duration of treatment with andrographolide.

CR10: I think, about this result, I would see possible result 10 partially support my hypothesis. This result may be due to an error in the experimental procedure and the experiment should be repeated several times to further ensure the accuracy of the results.

CR11: I think, about this result, I would see possible result 11 partially support my hypothesis. Despite the effect of andrographolide on the increase of p21 and p27, the increase of p21 and p27 has no effect on cell senescence and cell viability.

CR12: I think, about this result, I would see possible result 12 partially support my hypothesis. Andrographolide may act on cells through other pathways thereby causing cellular senescence, whereas andrographolide has no effect on p21 and p27.

CR13: I think, about this result, I would see possible result 13 partially support my hypothesis. Andrographolide has little effect on increasing p27, while increasing p21 might be useless in inducing senescence as well as cell death.

CR14: I think, about this result, I would see possible result 14 partially support my hypothesis. Andrographolide has little effect on increasing p21,while increasing p27 might be useless in inducing senescence as well as cell death.

CR15: I think, about this result, I would see possible result 15 partially support my hypothesis. Andrographolide induce cell death in other ways. For example, Andrographolide may cause direct cell death through chemical stimulation

CR16: I think, about this result, I would see possible result 16 fully contradicts my hypothesis.

There is a limitation in the current experiment: the experiment has not been conducted in mammals. To further ensure the accuracy of the experiments, we will conduct the experiments in mice with different Andrographolide concentration gradients and different treatment durations.

5. Conclusions

In conclusion, this study explores whether Andrographolide, a diterpene lactone from the Traditional Chinese Medicine Andrographis paniculate, induces senescence in colon cancer HCT-116 cells via p21 and p27.The result of the study would indicate whether Andrographolide induces senescence in colon cancer cells via p21 and p27.By conducting this experiment, the result would provide a theoretical support for follow-up studies in the treatment of colon cancer using Andrographolide.

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