The role of mechanical channel in the proliferation and migration of colon cancer

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Abstract. Piezo channel is the first family of mechanically gated cation channels found in mammals and has been shown correlated with the proliferation and metastasis of tumors in recent years. In a variety of cancer tissues, colon cancer tissues are particularly affected by mechanical stimulation. In this paper, we reported that the role of piezo1 in the proliferation and migration of colon cancer. Firstly, bioinformatics indicated that high levels of piezo1 expression existed in CRC tissues and were associated with poor prognosis. In vitro experiments, SW1116 cells were cultured in DMSO solutions, and CCK-8 assay and transwell assay were separately performed. It turned out that the inhibition of PIEZO1 by Dooku1 resulted in diminished cell proliferation and metastasis, while the activation of PIEZO1 by Yoda1 led to enhanced proliferation and migration of colon cells. Collectively, these findings demonstrated the facilitation role of piezo1 in colon cancer proliferation and migration, suggesting that piezo1 has the potential as a novel therapeutic target for drug design and treatment of colon cancer.

Keywords: Piezo1, Dooku1, Yoda1, mechanical channel, colon cancer, proliferation, migration.

1. Introduction

Mechanical channel is an ion channel that can sense the change of mechanical force of cell membrane and react quickly. Such reaction of ion channel can convert the mechanical signal felt by the membrane into electrical signal or chemical signal1. Among them, mechanically gated Piezo channel, including Piezo1 and Piezo2, is the first family of mechanically gated cation channels found and established in mammals. Piezo channel protein was found by Coste and Patapoutian who applied RNA interference technology to Neuro2A mice in 20102. Piezo1 is distributed in lung, bladder, skin, and other organs, also participates in blood vessel development, and osmolar homeostasis in red blood cells, regulates stem cell division and tumorigenesis3456. Piezo2 is distributed in dorsal root ganglion and related with pain-sensing, respiration, blood pressure, heart rate and so on. Piezo1/Piezo2 are also involved in bone formation and repair. In recent years, studies have shown that piezo1 plays an important role in a variety of malignant tumors, and mediates the proliferation, migration, and invasion of cancer cells through a variety of mechanisms7.

Colon cancer is a common malignant tumor in gastrointestinal tract and has been the third leading cause of cancer death with more than 930,000 deaths worldwide in 20208. Colon cancer cells are affected by four kinds of mechanical stimulation, and its proliferation and migration were more affected by mechanical stimulation than other common solid tumors9. Bioinformatics data shows the expression of ion channels in colon cancer tissues and adjacent tissues is different, and the prognosis of patients with low expression is better. In addition, elevated expression of Piezo1 promoted the proliferation and invasion of colon cancer cells9. In vitro, knockdown of Piezo1 resulted in reduced proliferation and migration of colon cancer cells10.

These studies reveal the regulatory role of Piezo1 in colon cancer, and its potential as a biomarker and therapeutic target for colon cancer treatment. Thereby it can be proposed that piezo1 inhibitors could inhibit the proliferation and migration of colon cancer cells, promote their apoptosis, and have the potential to become a medicine. Dooku1 is a PIEZO1 ion channel inhibitor which can antagonize Yoda1-evoked activation of Piezo111. Therefore, the study is intended to explore the anti-tumor effect of Dooku1 on colon cancer cells and its potential mechanism through vitro experimental study.

This paper reported the role of the mechanical channel piezo1 in the proliferation and migration of colon cancer. When piezo1 receptor was activated by piezo1 agonist Yoda1, the cultured colon cancer cells had higher proliferation and metastasis ability than the DMSO control group, while cultured by piezo1 antagonist Dooku1, colon cancer cells SW1116 had less proliferation and migration ability than the control. Collectively, these findings suggest that PIEZO1/2 has a regulatory role in COAD development, which can serve as an innovative therapeutic target for the prognosis and treat cancer.

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2. Materials & methods

2.1 Cells, reagents, and cell culture

The CRC cells were SW1116 cells from ATCC (American type culture collection). The cells were cultured in DMEM medium (SH30084.03, Hyclone, USA) containing 10% fetal bovine serum and 1% penicillin / Streptomycin at 5% CO2 and 37 ℃. When the degree of cell fusion was 80% ~ 90%, the cells were digested with trypsin, centrifuged, and subcultured.

2.2 CCK-8 assay

When the SW1116 cells were in logarithmic growth period, they were digested and centrifuged, and evenly inoculated into 96 well plates according to the density of 3000 / well and cultured in an incubator overnight. After the cells adhered to the wall, the concentrations of 1.0 μ mol · L-1 Dooku1 or Yoda1 or DMSO were added respectively. 6 repeat wells were set for each reagent. After incubating for 24 hours, 10 μL CCK-8 reagent were added to each well. continue incubating for 2 hours. The absorbance of cells at 450 nm wavelength was detected by enzyme labeling instrument.

2.3 Transwell assay

The SW1116 cells were cultured with medium containing 1.0 μ mol L-1Dooku1 or Yoda1 for 24 h, digested, centrifuged, suspended with serum-free medium. Then they were inoculated in the chamber at the concentration of 20 000 cells / well, each lower chamber containing 600 μ L of 10% fetal calf serum. The cells were incubated in a constant temperature incubator for 24 h, 4% paraformaldehyde fixed at room temperature for 15 min, 0.05% crystal violet stained for 15 min. The uncoated cells in the chamber were gently wiped, washed in PBS, and photographed under the microscope.

2.4 Data resources and Statistical analysis

The TCGA colon cancer tumor tissues and paired normal tissue samples' piezo1 gene expression and clinical data were downloaded from GEPIA12.

Statistical analysis was performed using GraphPad prism 8.0. Measurement data were expressed as mean ± standard deviation (x ± s.d.), with t-test and P <0.05 indicating a statistically significant difference.

3. Results

3.1 Expression differential analysis and survival analysis of piezo1

To find out if piezo1 has any effect on COAD development, we got the bioinformatics data of piezo1 expression in COAD cells and normal cells from database TCGA. From Figure 1a, the expressions of PIEZO 1 in COAD and normal tissue are found significantly different. The expression of piezo1 in tumor tissues is higher than adjacent tissues. This suggests a possible correlation between piezo1 and COAD development.

In survival analysis (Figure 1b), there is a negative correlation between piezo1 expression and patient prognosis. Among the COAD patients from TCGA database, the ten-year survival rate of COAD patients with low PIEZO1 transcripts per million (TPM) is 56%. In contrast, the ten-year survival rate of COAD patients with high PIEZO1 TPM is only 35%. Collectively, this information suggests that piezo1 may influence the proliferation and migration of colon cancer.

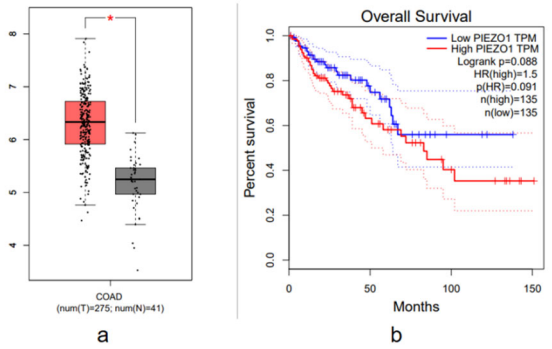

Figure 1. a. Piezo1 expression level in tumor and paired normal tissues (the ordinate is log2(TPM + 1), red=tumor, grey=normal); b. survival rates with different Piezo1 expression

3.2 Dooku1 inhibits the proliferation of COAD cells

Dooku1 is a small-molecule antagonist of piezo111. To determine the relationship between piezo1 and the proliferation of colon cancer cells, we used Dooku1 to inhibit PIEZO1 receptor in colon cancer cell SW1116. The experimental group was treated with Dooku1 in DMSO, while the control group was not treated with Dooku1, and the cell number was measured by CCK-8 assay (Figure 2a). The ratio-paired T-test for the experimental results showed that p =0.0191<0.05, so the result was statistically significant. The results showed that cell viability and proliferation were inhibited, cell apoptosis was increased, and the inhibitory effect was obvious in the first 72 hours.

3.3 Yoda1 promotes the proliferation of COAD cells

Yoda1 has been confirmed as a specific tool for activating piezo1 ion channels in various cells and tissues13. We examined the effects of Yoda1 on cell viability in the SW1116 cell using the CCK-8 assay (Figure 2b). The ratio-paired T-test for the experimental results showed that p =0.0442<0.05, so the result was statistically significant. The results showed that cell proliferation was promoted and cell apoptosis was decreased, and the promoting effect increased with time.

3.4 Dooku1 inhibits the metastasis of COAD cells

A transwell assay was performed to investigate whether Piezo1 was associated with colon cancer metastasis. Compared with the control group, cells treated with the piezo1 channel blocker Dooku1 migrated significantly

less, and cell metastasis was inhibited (Figure 2c & Figure3). The unpaired Welch T-test for the experimental results showed that p =0.0017<0.05, so the result was statistically significant.

3.5 Yoda1 promotes the metastasis of COAD cells

The transwell assays showed that compared with the control group, SW1116 cells treated with Piezo1 channel activator Yoda1 exhibited significantly increased cell migration and enhanced cell migration in the DMSO group (Figure 2d & Figure3). The unpaired Welch T-test for the experimental results showed that p =0.0046<0.05, so the result was statistically significant. These results suggest that PIEZO1 promotes colon cancer cell metastasis in vitro.

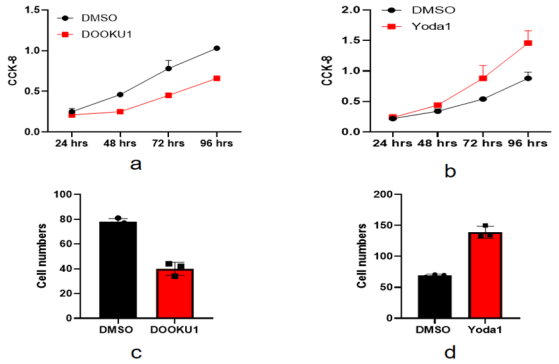

Figure 2.a, b. cell count of Dooku1, Yoda1 and their control groups; **c, d.** migrated cells of Dooku1, Yoda1 and their control groups

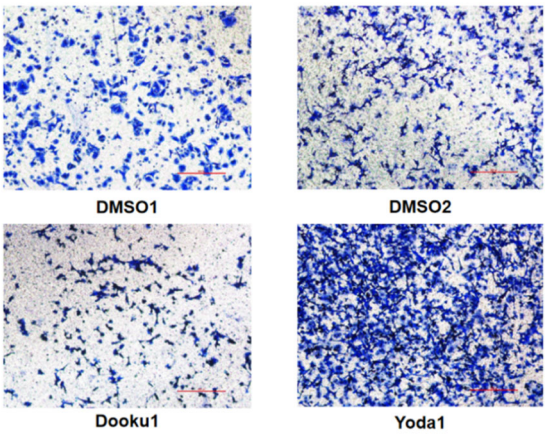

Figure 3. the result of transwell assay

4. Discussion

In summary, this paper reported the facilitation role of piezo1 in the proliferation and migration of colon cancer. The expression of piezo1 in tumor tissues is higher than adjacent tissues, and the prognosis of patients with low expression is better. When piezo1 was activated by agonist Yoda1, the proliferation and metastasis ability of colon cancer cells was enhanced. Colon cells cultured by piezo1 antagonist Dooku1 had less proliferation and migration ability than control group.

Piezo ion channels are selectively permeable for Na+, K+, Ca2+ and Mg2+. Based on the latest PIEZO1 structure and mechanism study141516, the researchers proposed the mechanism of mechanical force sensing of PIEZO: PIEZO forms a trimer, three-bladed propeller ion channel, with a central channel responsible for ion permeation, and a periphery of three blades responsible for mechanical force sensing. When the pore was closed, the blades embedded in the membrane were highly bended to the outside of the cell, indicating that they could bend the membrane and form a nanobowl-like Piezo-lipid membrane system. Under stress, as the cell membrane tension changes, PIEZO can change from curved to flat, opening the intermediate ion channels and converting mechanical force stimulation into cation flow17.

Functional studies suggest that Yoda1 can specifically but gently activate Piezo1 in mice and humans, but its precise interaction patterns are not confirmed18. Previous research showed that Piezo1 promotes colon cancer cell viability, migration, and metastasis, and could be involved in a possible regulatory mechanisms of Piezo1-MCU-HIF-1α-VEGF in colon cancer19. Our study is consistent with it. Dooku1 is a PIEZO1 ion channel inhibitor which can antagonize Yoda1-evoked activation of Piezo1. Despite previous study shows that Dooku1 does not affect Piezo1 constitutive activity and only antagonize Yoda1-evoked activation of Piezo111, our study still shows that Dooku1 inhibits the proliferation and metastasis of COAD cells. The reasons behind this are worth further studying.

Besides, the next step is to conduct an animal experiment instead of vitro cells. DMSO solution with Dooku1 and Yoda1 should be intravenously injected respectively into mice with COAD tumor formed previously, to observe the size and spread of COAD tumor so that the effect of PIEZO1/2 on COAD cells can be confirmed in vivo. Furthermore, because Yoda1 can activate both piezo1 and piezo2, another question is whether piezo1 or piezo2 participate in the regulation. Mice with gene for PIEZO1/2 molecules separately knocked out will be used in the above experiment so that the different impacts of PIEZO1 and PIEZO2 on COAD development can be determined.

Piezo1 can serves as a biomarker for cancer diagnosis and prognosis and a promising target for cancer therapy. Our study suggests a new strategy utilizing piezo receptors for the treatment of colon cancer.

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