Role of Notch1/2 signaling pathway in the apoptosis process of SGC-7901 induced by Oxaliplatin

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ABSTRACT

Objective: To explore the role of Notch1/2 signaling pathway in the apoptosis process of SGC-7901 induced by oxaliplatin. Methods: The cell viability was detected by CCK8 and the expression of Notch1/2 and Caspase9 was detected by Western Blotting before and after treatment of oxaliplatin. Results: Oxaliplatin medication decreased the viability of SGC-7901 and increased the Notch1/2 as well as Caspase9 expression. Notch signaling pathway inhibitor L685458 normalized those abnormalities greatly. Conclusion: Oxaliplatin promotes SGC-7901 apoptosis by activating Notch signaling pathway and up-regulating Caspase9 protein.

1. Introduction

Gastric cancer tops the malignant tumors in China, and it seriously impacts on patients’ health and life quality[1–3]. The main manifestations are digestive symptoms. It is easily to be transferred via direct infiltration, hematogenous metastasis, lymphatic metastasis, etc. The pathogenesis is related with various factors, such as Helicobacter pylori infection, genetic factors and gene mutation. However, the exact mechanism is still unclear[4–6]. Oxaliplatin has inhibitive effect on several carcinoma models mainly via inhibiting DNA synthesis[7,8]. It is effective on treating metastatic colon carcinoma, and is used as adjuvant therapy for III staging colon carcinoma after radical resection. In this study, we analyzed the role of Notch1 signaling pathway in induction of gastric cancer due to oxaliplatin.

2. Materials and methods

2.1. Instrument and reagents

Gastric cancer cell SGC7901 was provided by institution. Antibodies of Notch1/2 and caspase 9 were purchased from Abcam Company, USA. Second antibody and developing kit were purchased from Boster Lt. Company. Protein electrophoresis apparatus and Trans-Blot were purchased from Bio Rad Company. Centrifuge apparatus was purchased from Beckman Company.

2.2. Cell viability detection by CCK8 method

Cultured SGC7901 cells were inoculated in 96-hole culture plate, and were cultured in cell incubator. Then corresponding drugs were added in cells for incubation. Culture solution was abandoned, and the rest were rinsed by sterile PBS. MTT was added (5 mg/mL) in incubation for 4 h. Then 150 μL dimethyl sulfoxide was added in each hole. They were vibrated and dissolved. Absorbance was measured by microplate reader. The viability was calculated as follows: viability = (Absorbance control group- Absorbance observation group)/ Absorbance observation group 100%.
2.3. Notch signaling pathway related protein expression detected by Western Blotting

After culture, SGC7901 cells were rinsed by sterile phosphate buffer solution thrice. Then they were homogenated under ice bath. After protein quantification by Kjeldahl method, gel electrophoresis was performed with 10% hexadecyl sulfonate polyacrylamide and membrane transfer was performed with Semi-dry method. They were transferred to nitrocellulose membrane, and blocked with 4% defatted milk for 3 h. Then they were incubated with first antibody overnight. They were rinsed with sterile phosphate buffer solution thrice, and reacted with HRP-labeled secondly antibody for 1 h. The developing was performed. And the optical density was measured with gel imaging analyzer. GAPDH was used as β-actin to calculated the expression.

2.4. Statistical analysis

All data were analyzed by SPSS 16.0. Measurable data were analyzed by One-way analysis of variance, and were expressed as mean±SD. Enumeration data were analyzed by Chi-square test. The difference was significant as \( P<0.05 \).

3. Results

3.1. Inhibitive effect of oxaliplatin on viability of SGC–7901 cells

It showed that after incubation with oxaliplatin, the viability of SGC-7901 cells was significantly decreased. However, L685458, a inhibitor of Notch signaling pathway, could significantly resume the viability (\( P<0.01 \)) (Figure 1).

![Figure 1. Inhibitive effect of oxaliplatin on viability of SGC–7901 cells.](image)

**\( P<0.01 \), compared with control group; ###\( P<0.01 \), compared with oxaliplatin group.

3.2. Effect of oxaliplatin on Notch1 protein

It showed that after incubation with oxaliplatin, the expression of Notch1 protein was significantly increased. And L685458 could significantly resume the expression (\( P<0.01 \)) (Figure 2).

![Figure 2. Effect of oxaliplatin on Notch1 protein.](image)

**\( P<0.01 \), compared with control group; ###\( P<0.01 \), compared with oxaliplatin group.

3.3. Effect of oxaliplatin on Notch2 protein

It showed that after incubation with oxaliplatin, the expression of Notch2 protein was significantly increased. And L685458 could significantly resume the expression (\( P<0.01 \)) (Figure 3).

![Figure 3. Effect of oxaliplatin on Notch 2 protein.](image)

**\( P<0.01 \), compared with control group; ###\( P<0.01 \), compared with oxaliplatin group.
3.4. Effect of oxaliplatin on caspase 9 protein

It showed that after incubation with oxaliplatin, the expression of caspase 9 protein was significantly increased. And L685458 could significantly resume the expression ($P<0.01$) (Figure 4).

![Figure 3](image)

Figure 3. Effect of oxaliplatin on caspase 9 protein.  
**$P<0.01$, compared with control group; ## $P<0.01$, compared with oxaliplatin group.

4. Discussion

The morbidity and mortality of gastric cancer are increasing year by year. It has become serious threat to human life. The male are more susceptible to gastric cancer, with higher morbidity. Surgery is the main treatment, such as resection of neoplastic foci and radical surgery which resect all possible infiltrating peripheral tissues[9,10]. Chemotherapy is always used before, during and after radical surgery to prolong the survival time. Oxaliplatin has extensive clinical application, and has satisfactory effect. But the exact molecular mechanism of oxaliplatin is still unclear. We found in our study that oxaliplatin can significantly decrease viability of gastric cancer cells SGC-7901, up-regulate Notch1/2 protein and Caspase 9 protein expression; while L685458, a inhibitor of Notch signaling pathway, could significantly resume these abnormity. So oxaliplatin can promote apoptosis of SGC-7901 cells, in which Notch1/2 signaling pathway takes part. Notch signaling pathway participates in pathogenesis, development and metastasis of various tumors, which has been proved by many studies on anti-tumor drugs. Researches on correlation between osteosarcoma and proliferation and differentiation of hBMSC show that osteosarcoma cell U2OS can significantly promote the expression of Notch1/2/3, which is upstream protein of hBMSC, then can inhibit osteoblastic differentiation[11]. Study on expression of Notch in ovarian epithelial tumor also shows that the expression of Notch1 is related with clinical staging and differentiation degree, the higher the differentiation degree is, the higher the expression level of Notch1. It indicates that Notch1 has oncogene-like effect in pathogenesis and development of ovarian cancer[12]. The study on lymphoma cell of NK cell also shows that expressions of Notch 1, 3, 4 signaling molecule are significantly strengthened, indicating that Notch signaling pathway related protein takes part in the abnormality of tumor cells[13]. We also find that oxaliplatin significantly reduces the viability of gastric cancer SGC-7901, and upregulate the expression of Notch1/2 protein. And Notch pathway inhibitor, L685458 can significantly restore such abnormality. It suggests that abnormal Notch pathway takes part in the inhibited proliferation process by oxaliplatin. Cell apoptosis is programming death of cells, and is closely related with inhibition of various tumors. It is demonstrated that Notch signaling pathway has correlation with apoptosis[14,15]. Notch signaling pathway is found in DAPT induced apoptosis of esophageal squamous cell and apoptosis of human gastric cancer cells BGC-823. We also find that the expression of apoptosis protein Caspase 9 is strengthened, and Notch signaling pathway inhibitor can restore such abnormality, which indicates that Notch signaling pathway takes part in apoptosis of SGC-7901 cell. So induction by oxaliplatin can significantly promote apoptosis of SGC-7901 cell, and activated Notch 1/2 takes part in this pathological process.

References


