

Original Article

Molecular mechanism of ERK signal pathway in cell autophagy

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Abstract: Gastric carcinoma has unknown pathogenesis mechanism. Abnormal autophagy of cells is closely correlated with tumors such as gastric cancer. This study investigated pathogenesis mechanism of ERK signal pathway in gastric cancer occurrence and potential values in clinics. Using gastric carcinoma cell line SGC-7901 as the model, cells were treated by rapamycin, followed by Western blot to test autophagy and ERK signal pathway activation. ERK signal pathway agonist PMA and inhibitor U0126 were used to pre-treat SGC-7901 cells, whose autophagy and ERK signal pathway activation were tested. The correlation between ERK signal pathway and cell autophagy was investigated. Rapamycin-treated gastric cancer cells SGC-7901 led to cell autophagy and activation of ERK signal pathway. Treatment using ERK agonist PMA or antagonist U0126 enhanced or inhibited rapamycin-induced autophagy. The activation of ERK signal pathway in gastric carcinoma tissues with different progression stages was significantly correlated with autophagy level. Rapamycin may induce autophagy of gastric carcinoma cells directly or indirectly via ERK signal pathway, indicating that modulation of ERK signal pathway activation might be one potential strategy for treating gastric cancer.

Keywords: Rapamycin, ERK signal pathway, gastric carcinoma cells, cell autophagy

Introduction

Gastric carcinoma is one major disease in digestive system, with rapidly increasing incidence as life style transition [1]. Therefore, it is necessary to investigate pathogenesis mechanism of gastric carcinoma in order to provide new insights for clinical treatment. However, pathogenesis mechanism of gastric carcinoma is still unclear, as chemical reagents benzopyrene, chronic gastric ulcer, ionizing radiation, viral carcinogen, inflammation and high dosage chemotherapy all can lead to pathogenesis [2-4]. Clinical strategy for gastric cancer treatment is early diagnosis and treatment. Although relative satisfactory efficacy has been obtained, classical approaches of chemo- or radio-therapy frequently lead to internal bleeding, immune suppression, and dazzle or other side effects [5]. The improvement of accuracy and successful rate is one major challenge for both researchers and clinicians, making precise medication as the promising future perspective of gastric

cancer [6, 7]. However, the selection of target site is necessary for clinical treatment. More importantly, there is still no available treatment for gastric cancer using ERK signal pathway as the targeting site [8, 9].

Autophagy is one self-protection mechanism of body under stress condition [10, 11]. Under extreme conditions such as the threatening of life, cells can automatically degrade their own proteins or organelles [12, 13], thus exerting protective effects. ERK signal pathway is widely studied regarding its role in facilitating autophagy [14-16]. Currently there are few medicines targeting rapamycin, which is one protein of targeting ERK signal pathway. Moreover, decreasing ERK signal pathway protein expression obtained unsatisfactory effects [17, 18]. This study also examined potential molecular target of ERK signal pathway.

ERK signal pathway has wide functions. For example, it can suppress growth of gastric can-

Signal pathway of autophagy

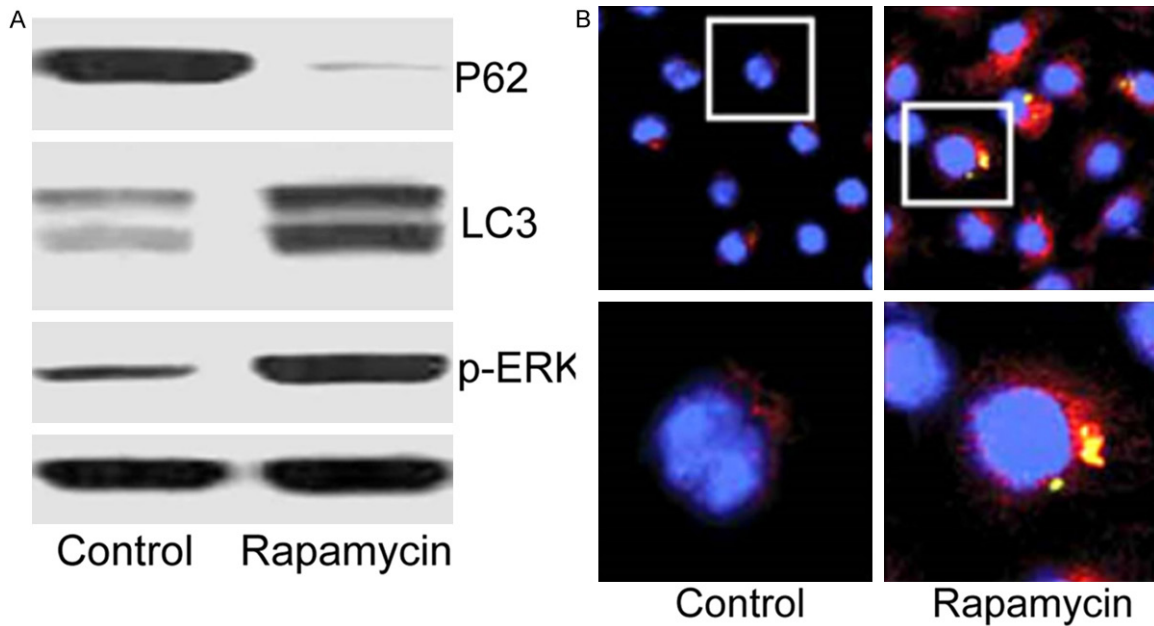


Figure 1. Rapamycin-treated SGC-7901 cells showed autophagy and ERK signal pathway activation. A. Western blot results for cell autophagy; B. Confocal microscopy images showing cell autophagy, blue, DAPI for nucleus, red, LC3 antibody for cell autophagy.

cer cells, and is related with tumor metastasis [10]. These results indicate the possible involvement of ERK signal pathway in onset and progression of gastric cancer [11]. This study utilized gastric cancer cell line SGC-7901 as the model, on which possible regulatory role of rapamycin was investigated.

This study discussed regulatory mechanism of cell autophagy, in order to provide evidences for searching targets of gastric cancer treatment.

Materials and methods

Reagents and cell model

Gastric carcinoma cell line SGC-7901 was purchased from American Microbial Strain Center (Virginia, US).

Fetal bovine serum (FBS) and cell culture medium were purchased from Hualan Bio (China). Other common reagents were purchased from Santa Cruz (US). Test kit for cell autophagy was purchased from Beyotime (China).

Cell culture

SGC-7901 cells were cultured by normal methods [19].

Assay for activity of ERK signal pathway

ERK signal pathway activity inside gastric carcinoma cell line SGC-7901 was tested using test kit as routine methods described [20]. In brief, cells were cultured and treated by rapamycin as described above. Cell activity assay buffer (containing 2 mg/ml ERK signal pathway activity assay reagent) was purchased from Santa Cruz, US. Gastric carcinoma SGC-7901 cells were cultured for 4 h, followed by the addition of DMSO to quench the reaction in 5 min incubation. SGC-7901 cells incubating in 24-well plate was then loaded onto a microplate reader for measuring absorbance values at 560 nm. Growth curve of cells was plotted [21].

Western blotting for cell autophagy

SGC-7901 cells treated with rapamycin or ERK signal pathway inhibitor U0126/activator PMA (Santa Cruz, US) was extracted for protein suspensions using extraction kit (Santa Cruz, US) following manual instruction. Protein concentration was quantified. Cell lysate was extracted and quantified in a microplate reader. Proteins were separated by centrifugation. Equal volume of cell protein suspensions (20 μ g) were boiled in water-bath, followed by Western Blot. Primary antibody was added for

Signal pathway of autophagy

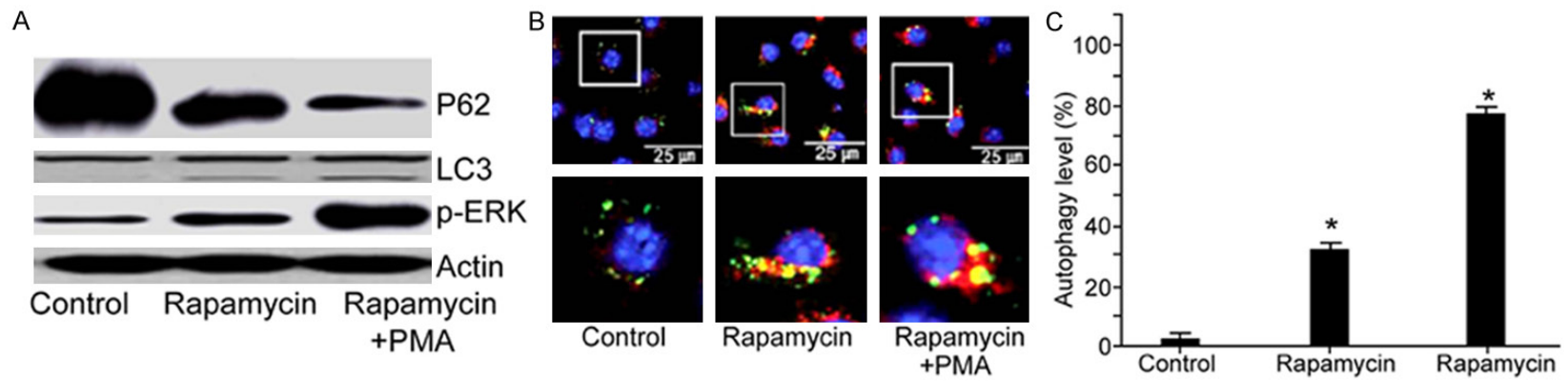


Figure 2. ERK signal pathway activator PMA enhanced rapamycin-induced SGC-7901 cell autophagy. A. Western blot for cell autophagy; B. Confocal microscopy images showing cell autophagy, blue, DAPI for nucleus, red, LC3 antibody for cell autophagy; C. Statistics for cell autophagy.

Signal pathway of autophagy

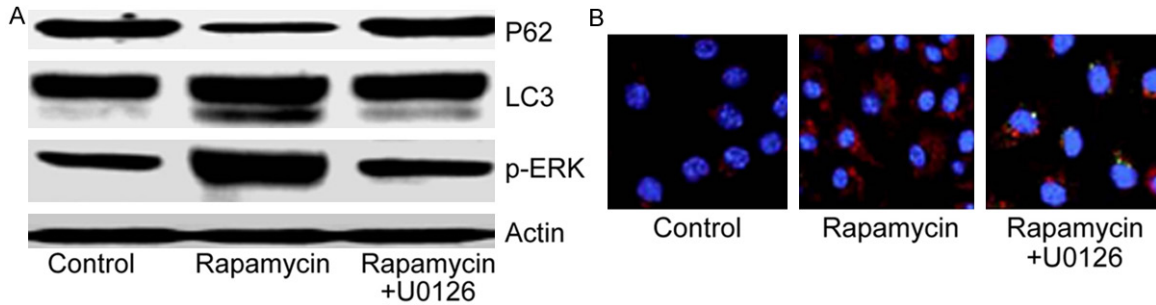


Figure 3. ERK signal pathway inhibitor U0126 decreased rapamycin-induced SGC-7901 cell autophagy. A. Western blot for cell autophagy; B. Confocal microscopy images showing cell autophagy, blue, DAPI for nucleus, red, LC3 antibody for cell autophagy.

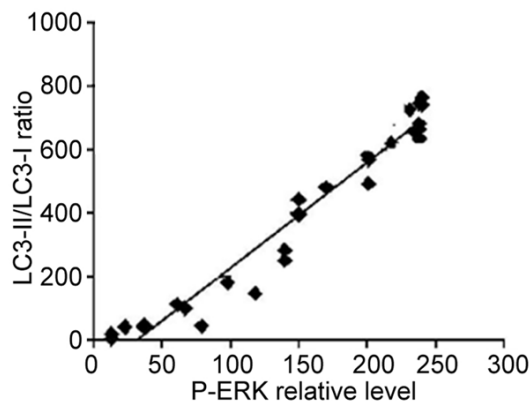


Figure 4. Correlation analysis between ERK signal pathway activation and autophagy level.

4°C overnight incubation (1:1000) dilution. After TBST washing, primary antibody was removed, followed by the addition of secondary antibody (1:2500). After incubation at 37°C for 2 h, TBST was added for washing, and ultra-sensitive reagent was used for membrane development. By analyzing anti-ERK level, anti-mouse IgG antibody was added for incubation, followed by three times of rinsing. Horseradish peroxidase was added for staining. Gel imaging system was used for quantifying proteins by analyzing gray values. A gel imaging system (Qinxiang, China) was used to analyze protein expression level, for comparing ERK signal pathway expression level in SGC-7901 cells [22].

Immunofluorescence analysis for cell autophagy

SGC-7901 cells were cultured by rapamycin or ERK signal pathway inhibitor/activator pre-treatment. After fixation, blocking and permea-

bilization, cell autophagy was tested by fluorescent kit.

Statistical analysis

SPSS15.0 was used for statistical analysis. Student t-test was used for comparing between groups of SGC-7901 cells. A statistical significance was defined when $P < 0.05$ in replicated experiments.

Results

Rapamycin-treated SGC-7901 cells showed autophagy and ERK signal pathway activation

As shown in **Figure 1**, rapamycin plus UV radiation on gastric cell line SGC7901 cells led to ERK signal pathway activation, as gray values of p-P38 activity in rapamycin treatment group by Western blot increased to 7.2 ± 0.4 folds of that in control group. Moreover, rapamycin treatment elevated cell autophagy from almost 0 to $79.7\% \pm 3.5\%$.

ERK signal pathway activator PMA enhanced rapamycin-induced SGC-7901 cell autophagy

As shown in **Figure 2**, ERK signal pathway agonist PMA enhanced autophagy of SGC-7901 cells after pre-treatment by rapamycin by about 37%.

ERK signal pathway inhibitor U0126 decreased rapamycin-induced SGC-7901 cell autophagy

As shown in **Figure 3**, ERK signal pathway inhibitor U0126 suppressed rapamycin-induced SGC-7901 cell autophagy.

Signal pathway of autophagy

ERK signal pathway activation and its correlation with autophagy level

As shown in **Figure 4**, ERK signal pathway activation level across gastric carcinoma tissues with different grades were positively correlated with autophagy level ($R=91.3\%$, $P=0.0037$).

Discussion

Gastric carcinoma is one major tumor in digestive tract. The investigation for its molecular mechanism thus has important values. How to improve accuracy and successful rate of gastric carcinoma is thus one major challenge currently. Precise medication is one effective method targeting gastric cancer [6, 7]. However, the selection of targets is the most difficult part. Clinical treatment thus necessitates more effective molecular targets for gastric carcinoma. More importantly, no available treatment has been developed targeting ERK signal pathway [8, 9].

This study utilized gastric carcinoma cell line SGC-7901 as the model, on which regulatory role of rapamycin and possible mechanisms were studied from both molecular and protein levels. Data showed that rapamycin enhanced autophagy of human gastric cancer cell line SGC-7901 autophagy, as consistent with previous results [23]. Abnormal cell autophagy is correlated with aging, neurodegenerative disease and gastric carcinoma. However, the signal transduction mechanism of autophagy in occurrence of gastric cancer is still unclear. This study thus investigated the potential usage of ERK signal pathway-mediated cell autophagy.

How rapamycin regulates gastric cancer cell growth and autophagy is still unclear [24]. ERK signal pathway can suppress growth of gastric cancer, whilst ERK signal pathway is correlated with tumor metastasis [25], indicating possible involvement of ERK signal pathway in onset and occurrence of gastric carcinoma [26].

ERK signal pathway participates in cell autophagy. In current research, whether ERK signal pathway is under regulation by rapamycin for further mediating autophagy of gastric cancer cell line SGC-7901, is still unknown [27, 28]. Result of this study showed that rapamycin enhanced ERK signal pathway level. After pre-treatment using ERK signal pathway activator,

autophagy rate of gastric cancer cell line SGC-7901 was elevated, whilst ERK signal pathway inhibitor U0126 suppressed rapamycin-induced SGC-7901 cell autophagy, indicating involvement of ERK signal pathway in regulating autophagy. Improvement of accuracy and successful rate of gastric cancer treatment is one major challenge in medicine. In clinics, precise medication is more effective for gastric cancer treatment [6, 7]. However, the selection of treatment target is the most difficult part. Therefore, more effective molecular target for gastric cancer is required. More importantly, currently no treatment has been developed targeting ERK signal pathway [8, 9].

In this study, the role of ERK signal pathway proteins in rapamycin-induced SGC-7901 cell autophagy can be demonstrated by three aspects of results. Firstly, rapamycin-treated SGC-7901 cells had significantly potentiated ERK pathway protein activity; secondly, after pre-treatment by ERK signal pathway activator, cell autophagy was potentiated by rapamycin induction; Thirdly, U0126 suppressed rapamycin-induced cell autophagy. ERK signal pathway protein plays roles in rapamycin-induced autophagy of SGC-7901 cells. Targeting ERK signal pathway might be one novel strategy for molecular treatment of gastric cancer [26]. Currently, ERK signal pathway also inhibits cancer cell autophagy in other cancers [28]. These results suggested that rapamycin could induce autophagy of gastric carcinoma cell line SGC-7901 cells via enhancing ERK signal pathway.

Certain weakness, however, existed in the currently study. Firstly, no clinical tumor tissues or adjacent tissues of gastric carcinoma were involved, thus lacking evidence linking ERK and gastric cancer. Secondly, gastric cancer tissues from patients post-treatment was also missing, making it inaccessible to investigate the correlation between ERK signal pathway and prognosis of gastric cancer. Thirdly, this study did not employ animal model for gastric carcinoma for rapamycin treatment *in vivo*, requiring treatment efficacy study for rapamycin in gastric cancer.

Conclusion

Rapamycin can regulate gastric cancer autophagy via ERK signal pathway, forming one possi-

ble molecular mechanism of gastric cancer. These results indicated that mediation of ERK signal pathway activation benefit the management of gastric cancer cell autophagy, forming one potential strategy for gastric cancer treatment.

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Disclosure of conflict of interest

None.

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