

Original Article

Knockdown of Aurora A suppresses cell proliferation of human gastric cancer cells by inhibiting cell autophagy

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Abstract: Background: Gastric cancer is one of the major causes of cancer-related death in the world. Aurora kinases are reported to be up-regulated in various cancers. In the present study, the human gastric cancer cells were interfered with Aurora A specific siRNAs. We explored the function of Aurora A and investigated the molecular mechanism in human gastric cancer cells. Methods: The levels of Aurora A were determined by Western blotting analysis. The expression of Aurora A was interfered with Aurora A specific siRNA in MKN-28 cells and BGC-823 cells. The proliferation of MKN-28 cells and BGC-823 cells was determined by MTT assay before and after Aurora A siRNAs transfection. Expression levels of LC3-I, LC3-II and p62 in BGC-823 cells were determined by Western blotting analysis before and after Aurora A siRNA or negative control siRNA transfection. Results: Aurora A was observed in human gastric cancer cell lines, including MKN-45 cells, BGC-823 cells and MKN-28 cells. The level of Aurora A was significantly higher in BGC-823 cells and MKN-28 cells than that in MKN-45 cells. The expression of Aurora A was significantly decreased in MKN-28 cells and BGC-823 cells by transfection with Aurora A siRNA. Knockdown the expression of Aurora A significantly suppressed the cell proliferation of MKN-28 and BGC-823 cells. Furthermore, the Western blotting analysis results demonstrated that knockdown Aurora A obviously promoted cell autophagy of human gastric cancer cells. Conclusion: All results demonstrated that knockdown Aurora A inhibited the cell proliferation of human gastric cancers, which was partly due to cell autophagy increased in Aurora A siRNA transfected gastric cancer cells.

Keywords: Aurora A, cell autophagy, gastric cancer, interference

Introduction

Gastric cancer is one of the easily seen diseases globally and responsible for a high burden of diseases [1, 2]. In recent years, the medical technology has been improved, including standardized surgery, chemotherapy, radiotherapy, immunized therapy or combination therapy for gastric cancers [3-5]. However, gastric tumor is still the main reason to affect human health due to its high morbidity and mortality [6-8]. It is urgent to find an effective way to cure gastric cancer and to identify a molecular marker on diagnosis of gastric cancers.

It is helpful in the therapy of human gastric cancers to clarify the molecular mechanism of gastric tumorigenesis. Aurora A, a serine/threonine-protein kinase in humans, is encoded by the AURKA gene, which plays an important role

during mitosis and meiosis of cell proliferation [9, 10]. Cancer is partly due to the malignant proliferation of normal cells, but the role of Aurora A has not clearly clarified in the tumorigenesis and proliferation of gastric cancer cells [11]. The researchers had detected the expression and prognostic significance of Aurora A in 89 gastric cancer patients treated with curative surgery. They found that Aurora-A was correlated with tumor progression ($P=0.053$) and shorter survival ($P=0.001$) and Aurora A was an independent unfavorable prognostic factor and identified patients with worse outcome even in a relatively early and local disease [12]. Moreover, Aurora A was deleted with Aurora A specific small interference RNA in GES-1, SGC-7901 cell lines and the results demonstrated that knockdown of Aurora A could inhibit cell growth and induce cell cycle arrest at G1 phase [13]. Lee group found that Aurora kinase inhibi-

tor decreased c-Myc expression in gastric cancer cells and recombinant human bone morphogenetic protein-2 (rhBMP-2) remarkably decreased the proliferation of gastric cancer cells via the inactivation of beta-catenin via c-Myc and AURKs [14]. Recently, Aurora A was reported to be involved in canonical Wnt signaling in gastric carcinogenesis [15]. Specifically, Aurora A had a strong correlation with Rac GTPase-activating protein 1 (RACGAP1), a modulator of the canonical Wnt signaling pathway, and inversely associated with CDKN1A expression [15].

Programmed cell death has occurred in progression of cancers, such as cell apoptosis, autophagy and necroptosis [16-18]. Autophagy has been reported to either inhibit or promote cell growth of cancer in tumorigenesis of various cancers [19-21]. In the present study, we explored the molecular mechanism on Aurora A and cell autophagy in human gastric cancer cells, which would provide helpful reference in the clinical therapy of human gastric cancers.

Materials and methods

Cell lines

The gastric cancer cell lines including MKN-28, BGC-823 and MKN-45 were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum (Invitrogen), 50 IU/ml penicillin, and 50 mg/ml streptomycin in a 5% CO₂ incubator at 37°C.

Cell transfection

ARK-1 siRNA (h): sc-29731 and Control siRNA-A: sc-37007 was obtained from Santa Cruz Biotechnology INC. The gastric cancer cells were transfected with ARK-1 siRNA and control siRNA by using lipofectamineTMRNAiMAX (Invitrogen) according to kit protocol. Briefly, the gastric cancer cells (5×10³ cell/well) were plated into 48-well plate and the cells were cultured in 500 µL of growth medium without antibiotics until they got 50% confluent before transfection. Then, 6 pmol RNAi duplex and 1 µL of lipofectamineTMRNAiMAX were diluted in 50 µL Opti-MEM I Reduced Serum Medium without serum and gently mixed, respectively. The diluted RNAi duplex and diluted lipofectamineTMRNAiMAX were gently mixed and incu-

bated for 15 min at room temperature. Then, the mixture was added into each well of the plate and the cells were incubated for 24 h, 48 h and 72 h at 37°C in a CO₂ incubator.

MTT assay

Cell viability was determined by MTT assay. The gastric cancer cells were transfected with Aurora A siRNA and control siRNA and incubated at 37°C in a 5% CO₂ incubator. The cells were incubated for 24 h, 48 h and 72 h, respectively. Before test, 10 µL of 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (5 mg/mL) was added into the medium for 4 hours. The absorbance of each well was read at 490 nm with a microplate reader.

Antibodies

Anti-Aurora A antibody [EP1008Y] was a recombinant rabbit monoclonal antibody (Cat. No. ab52973), which was diluted at the ratio of 1:10000. Anti-LC3 (Cat. No. L8918) was obtained from Sigma-Aldrich Corporation. The antibody recognizes human and rat LC3A/B-I and LC3A/B-II by immunoblotting and immunoprecipitation (~18 kDa and ~16 kDa, respectively), which was diluted at the ratio of 1:1000. Anti-p62 antibody (Cat. No. ab56416) was a mouse monoclonal to SQSTM1/p62 and the dilution is 1:1000. Anti-β-actin antibody was purchased from TransGen Biotechnology (Beijing, China).

Western blotting analysis

The human gastric cancer cells were lysed using a lysis buffer (Invitrogen). The total proteins were fractionated by using 12% SDS-PAGE and transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA) at 400 mA for 1 h. The membrane was blocked with 5% BSA in TBST buffer. Then the membrane was incubated with primary antibodies at 4 overnight and secondary antibodies for 30 min at room temperature. Finally, the bands were visualized by an enhanced chemiluminescence detection kit (Thermo Scientific Pierce ECL).

Statistical analysis

The results were analyzed by SPSS20.0 software (SPSS Inc., Illinois, USA). The tests used for statistical analysis in each experiment were

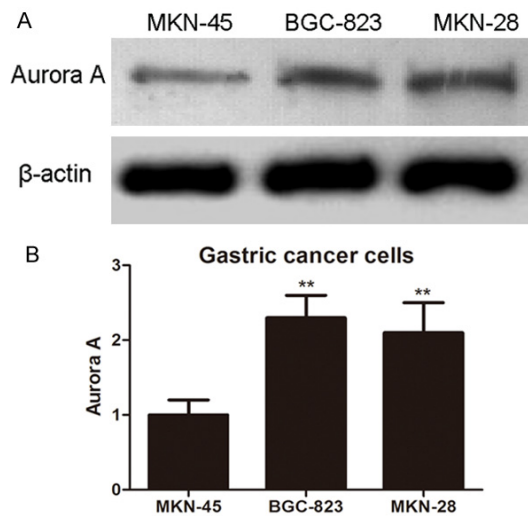


Figure 1. Aurora A is highly expressed in human gastric cancer cells. A. The gastric cancer cells were plated into 6-well plate. After cultured for 6 hours, the levels of Aurora A in MKN-45 cells, BGC-823 cells and MKN-28 cells were detected by western blotting analysis. In the present study, beta-actin was used as the internal reference gene. B. The levels of Aurora A were shown in histogram. Here, we used the level of Aurora A in MKN-45 cells as control. The expression levels of Aurora A in BGC-823 cells and MKN-28 cells were significantly increased, compared with that in MKN-45 (** $P < 0.01$).

two sets of independent samples t-test. All the data were shown as mean value \pm S.D. $P < 0.05$ was considered as statistically different.

Results

Aurora A is highly expressed in human gastric cancer cells

MKN-28 cells are high differentiated gastric adenocarcinoma cell line. BGC-823 cells and MKN-45 cells are the low differentiated gastric adenocarcinoma cell line. In the experiment, we detected the Aurora A levels in three gastric cancer cell lines including MKN-45, BGC-823 and MKN-28 by western blotting analysis. As shown in **Figure 1**, the expression levels of Aurora A were detected in three cell lines, and the Aurora A levels were significantly increased in BGC-823 cells and MKN-28 cells than that in MKN-45 cells (** $P < 0.01$).

The endogenous Aurora A expression is interfered with Aurora A siRNA

Next, the MKN-28 cells and BGC-823 cells were transfected with Aurora A siRNA and negative control siRNA for 48 hours. Aurora A lev-

els were interfered by Aurora A specific siRNA, and the expression level of Aurora A was detected by western blotting analysis. As shown in **Figure 2**, the Aurora A level was significantly decreased in Aurora A siRNA transfected-MKN-28 cells, compared with negative siRNA transfected MKN-28 cells (** $P < 0.01$). Furthermore, this was consistent with that in BGC-823 cells. The Aurora A siRNA and negative control siRNA was used to transfer into BGC-823 cells for 48 hours. The endogenous aurora A was significantly interfered in BGC-823 cells transfected with Aurora A siRNA than that transfected with negative control siRNA (** $P < 0.01$).

Knock down the Aurora A expression significantly suppresses the proliferation of human gastric cancer cells

In order to test the role of Aurora A in human gastric cancer cells, the Aurora A siRNA transfected MKN-28 cells and negative control siRNA transfected MKN-28 cells were cultured for 24 hours, 48 hours and 72 hours, respectively. The inhibition rate was determined by MTT assay. As shown in **Figure 3A**, the inhibition rate of Aurora A siRNA-transfected MKN-28 cells was significantly increased than that of negative control siRNA-transfected MKN-28 cells (** $P < 0.01$), which was consistent with the results in 72 hours.

This was also tested and confirmed in BGC-823 cells. The BGC-823 cells were transfected with Aurora A siRNA and negative control siRNA for 48 hours and 72 hours, respectively. Cell viability was determined by MTT assay. As shown in **Figure 3B**, the cell viability was significantly decreased in Aurora A siRNA-transfected BGC-823 cells than that of negative control siRNA-transfected cells (** $P < 0.01$, * $P < 0.05$). All the data obviously demonstrated that transfection of Aurora siRNAs significantly inhibited cell proliferation of human gastric cancer cells.

Knockdown of Aurora A obviously inhibits cell autophagy in BGC-823 cells

In order to further clarify the molecular mechanism of Aurora A in human gastric cancer cells, western blotting analysis was performed to test whether Aurora A was involved in cell autophagy in BGC-823 cells. As shown in **Figure 4A**, BGC-823 cells were transfected with Aurora A siRNAs for 48 hours, the levels of LC3-I and

Aurora A and gastric cancer

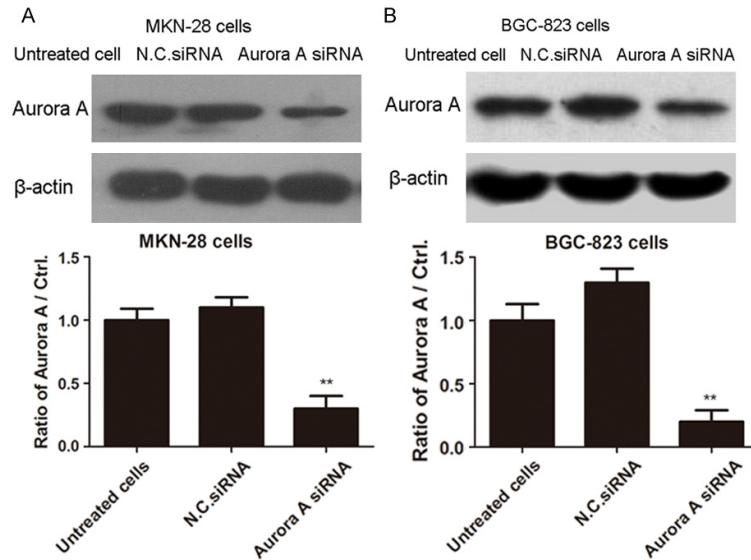


Figure 2. The endogenous Aurora A expression is interfered with Aurora A siRNA. The MKN-28 cells (A) and BGC-823 cells (B) were plated into 48-well plate and transfected with Aurora A siRNA and negative control siRNA for 48 hours. The untreated cells were used as negative controls. The expression levels of Aurora A were normalized to β -actin. The ratios of Aurora A in Aurora A siRNA-transfected MKN-28 cells and BGC-823 cells, negative control siRNA-transfected MKN-28 cells and BGC-823 cells were shown in histogram. ** $P < 0.01$, compared with negative control siRNA transfected cells.

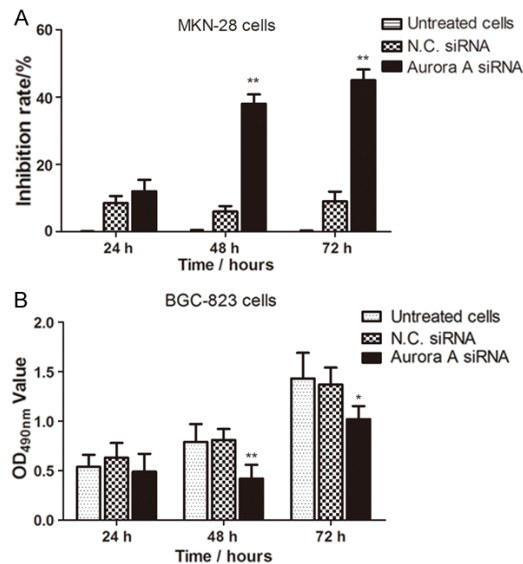


Figure 3. Knockdown the Aurora A expression significantly suppresses the proliferation of human gastric cancer cells. A. MKN-28 cells were transfected with Aurora A siRNA and negative control siRNA for 24 h, 48 h and 72 h, respectively. The cell viability was determined by MTT assay and inhibition rate was shown in histogram. ** $P < 0.01$, compared with negative control siRNA transfected MKN-28 cells. B. BGC-823 cells were transfected with Aurora A siRNA and negative control siRNA for 24 h, 48 h and 72 h, respectively. The cell viability was determined by MTT assay. * $P < 0.05$, ** $P < 0.01$, compared with negative control siRNA group.

LC3-II were obviously increased in Aurora A siRNA-transfected cells. Additionally, the level of p62 was obviously decreased in Aurora A siRNA-transfected cells (Figure 4B). All the data suggested that interference with endogenous Aurora A could suppress the cell autophagy in human gastric cancer cells.

Discussion

Gastric cancer is one of the deadly malignant tumors in the world [22]. In the present study, we interfered with the endogenous Aurora A in human gastric cancer cells with Aurora A specific siRNAs. The cell viability was significantly decreased in Aurora A siRNA-transfected gastric cancer cells. Interestingly, the western blotting analysis results demonstrated that knockdown

Aurora A remarkably increased the levels of LC3-II and decreased the expression of p62. All the results revealed that Aurora A-mediated cell autophagy contributed to the proliferation inhibition of human gastric cancer cells. The clear understanding of molecular mechanisms of gastric cancer may provide new clues in the clinical therapy against gastric cancers.

Firstly, western blotting analysis was performed to determine the expression of Aurora A in several gastric cancer cell lines, such as MKN-45, BGC-823 and MKN-28. The expression of Aurora A was remarkably observed in human gastric cancer cells. This was consistent and further confirmed with Katsha [23], in which they claimed that Aurora A was up-regulated during chronic inflammation, and the levels of Aurora A in premalignant and malignant lesions from gastric mucosa of patients were significantly increased compared with healthy gastric tissues.

Next, we used Aurora A specific siRNA to interfere with endogenous Aurora A in human gastric cancer cells and the levels of Aurora A were significantly decreased in BGC-823 cells and MKN-28 cells. Moreover, knockdown of Aurora A inhibited cell proliferation of human gastric

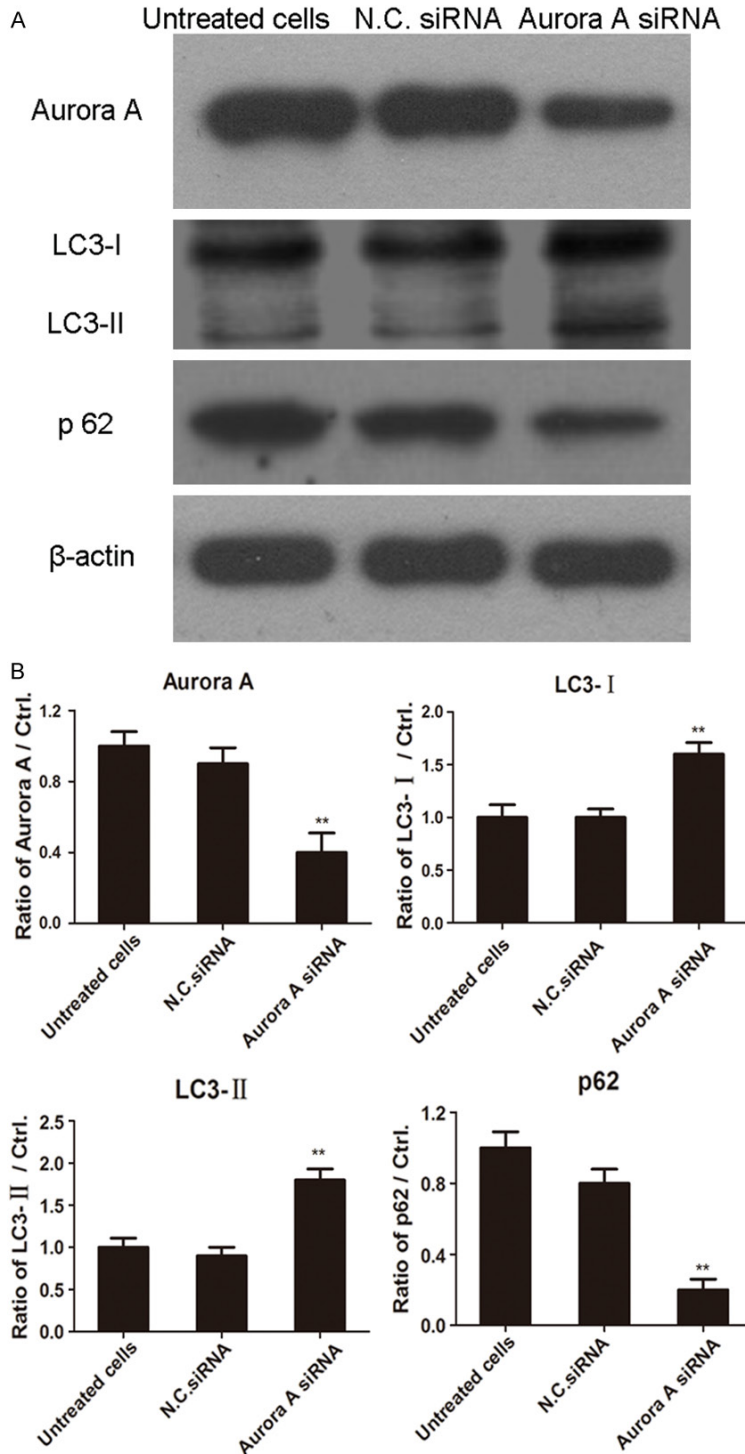


Figure 4. Knockdown of Aurora A obviously inhibits cell autophagy in BGC-823 cells. A. The human gastric cancer cells were plated into 24-well plate and transfected with Aurora A specific siRNAs and negative control siRNA for 48 hours. The levels of Aurora A, LC3-I, LC3-II and p62 were tested by western blotting analysis. Beta-actin was used as internal reference gene in the experiment. B. The level of LC3-II is increased and p62 level is obviously decreased. The proteins in autophagy were tested and the levels of Aurora A, LC3-I, LC3-II and p62 were shown in histogram. **P<0.01, compared with negative control siRNA transfected BGC-823 cells.

cancer cells, which was consistent with Wang, W. [13]. They found that deletion of Aurora A inhibited proliferation and induced G1 arrest suggesting Aurora A worked as an oncogene in human gastric cancers.

Furthermore, the molecular mechanism on Aurora A in gastric cancer cells was also clarified. Western blotting analysis results demonstrated that knockdown of Aurora A remarkably increased the levels of LC3 II and decreased the levels of its substrate, p62, suggesting Aurora A depletion might contribute to cell autophagy of gastric cancer and the Aurora A knockdown induced cell autophagy could probably promote cell death of gastric cancer cells. In conclusion, inhibition of Aurora A by specific siRNAs or Aurora A inhibitors might be an effective way to suppress the progression of gastric cancer.

Conclusions

All the results revealed that knockdown Aurora A inhibited the cell proliferation of human gastric cancers, which was partly due to cell autophagy was increased in Aurora A siRNA transfected gastric cancer cells.

Disclosure of conflict of interest

None.

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