RESEARCH COMMUNICATION

Inhibition of Proliferation, Viability, Migration and Invasion of Gastric Cancer Cells by Aurora-A Deletion

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Abstract

Accumulating evidence has demonstrated Aurora-A to be frequently overexpressed in many cancers, including gastric cancer. In order to study the effects of Aurora-A on gastric cancer cells, we detected the changes of cell phenotype after treatment with Aurora-A specific small interference RNA (siRNA). In addition, VX-680 was used simultaneously. RT-PCR and western-blot were used to determine the level of Aurora-A mRNA and protein in cells, including GES-1, SGC-7901, SGC-7901 lines treated with VX-680, SGC-7901 treated with DMSO, SGC-7901 interfered using siRNA and SGC-7901 interfered using scrambled RNAi. MTT, PI staining and transwell assays were used respectively to analyze proliferation, viability, and migration and invasion of the cells. The results showed that deletion of Aurora-A may inhibit proliferation and induce G1 arrest. The transwell assay indicated that Aurora-A may promote metastasis of gastric cancer. Collectively, our findings support Aurora-A as an oncogene in gastric cancer. Deletion of Aurora-A may have potential as a therapeutic method for gastric cancer.

Keywords: Gastric cancer cells - Aurora-A - proliferation - viability - VX-680

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Introduction

The aurora kinases are key mitotic regulators in eukaryotes (Meraldi et al., 2004). According to the distinctions of subcellular localization and function, aurora kinases have been classified into A, B, and C groups (Carmena and Earnshaw, 2003; Nigg, 2001). The Aurora-A gene locus is located in the 20q13 chromosome region and identified in many cancer cells for its overexpression such as breast (Sen et al., 1997), colon (Bischoff et al., 1998), pancreas (Li et al., 2003), prostate (Bar-Shira et al., 2002), liver (Jeng et al., 2004), ovarian (Moreno-Bueno et al., 2003), medulloblastoma (Neben et al., 2004), gliomas (Reichardt et al., 2003), stomach (Sakakura et al., 2001) and bladder cancers (Sen et al., 2002). Aberrant Aurora-A expression is associated with an increased risk of colon cancer (Bischoff et al., 1998). Its overexpression can lead rodent fibroblasts to immortalization (Zhou et al., 1998). A previous study has shown that expression of p53 can suppress functions of Aurora-A (Chen et al., 2002). Another study showed that Aurora-A can phosphorylate p53 at Ser315 and lead it to loss function (Katayama et al., 2004). All these evidence has accumulated to show that Aurora-A plays a role as an oncogene.

Not only in China but also in the world, gastric cancer is one of the commonest malignancies (Weidner, 1995; Xin et al., 2001). It is the second most common cause of cancer-related death in the world (Whelan, 1993). Its molecular mechanism remains unclear. However, multiple genes have participated in the progress of pathogenesis and development.

In the previous study, Aurora-A overexpression in gastric cancer has been identified. In this study, the effects of Aurora-A on gastric cancer cells was unraveled. By observing proliferation, viability, migration and invasion of gastric cancer cell, the results indicated deletion of Aurora-A may suppress pathogenesis and metastasis of gastric cancer.

Materials and Methods

Cell culture

GES-1 (gastric mucosa cell) and SGC-7901 (gastric cancer cell) were obtained from the American Type Culture Collection (Bethesda, MD, USA) and grown in RPMI-1640 medium (Hyclone, USA) supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 ug/ml streptomycin). Cells were maintained in a humidified cell incubator with 5% CO2 at 37 ºC.

RT-PCR

Total RNA was isolated using a RNeasy Mini Kit (Biomed, China). cDNA was reverse transcribed with 1 μg of total RNA, using TaKaRa Reverse Transcription Kit (TaKaRa, Japan). cDNA was amplified using the following primers. The Aurora-A primers were

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5'-AATGATTGAAGGTCGGATGC-3' (sense) and 5'-TTCTCTGAGCATTGGCCTCT-3' (antisense). The GAPDH was used as an internal control. The GAPDH primers were 5'-AGAAGGCTGGGGCTCATTTG-3' (sense) and 5'-AGGGGCCATCCACAGTCTTC-3' (antisense). PCR was performed for 35 cycles under the conditions of annealing at 58 ºC (30 s), extension at 72 ºC (30 s), and denaturing at 94 ºC (30 s) using a TaKaRa thermo cycler.

siRNA against Aurora-A

The oligonucleotide for Aurora-A RNAi is 5'-AACTGTGTCTCCAGGCCTG-3'. Scrambled RNAi: 5'-AGCCAAATGGCGGAAGCCA-3' was used as a control for the experiment. Cells were seeded onto 60 mm plates for 24 h and transfected with siRNA or control siRNA for 48 h using Lipofectamine 2000 (Invitrogen) according to manufacturer's instructions.

Drug treatment

VX-680 was purchased from Merck&Co Inc, and dissolved in dimethyl sulfoxide at a concentration of 2mM. 100 nM of the drug was used and incubated with cells for 48 h.

Antibodies and western-blot

Cells were lysed in RIPA (150 mM NaCl, 2 mM EDTA, 1% Nonidet P-40, 50 mM NaF, 1 mM Na3VO4, 1 mM Na2MoO4, 10 μM aprotinin and 10 μM leupeptin) on ice. The cells contained GES-1, SGC-7901, SGC-7901 treated with VX-680, SGC-7901 treated with DMSO, SGC-7901 interfered using siRNA and SGC-7901 interfered using scrambled RNAi. Crude cell lysates were centrifuged at 14,000 g for 10 min and cleared lysates were collected. Samples were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes. Membranes were blocked in 5% milk-TBST and then probed with anti-Aurora-A (Transduction, Germany). The reaction was followed by probing with peroxidase-coupled secondary antibodies and then detected by enhanced chemiluminescence (Amersham Pharmacia, USA). Anti-β-actin (Santa Cruz, Japan) used as an internal control.

Cell growth assay

Cell growth was determined by MTT assay. The cells as described above (1500 cells/well) were plated in 96-well plates and incubated with the normal conditions. Then the cells were treated with 10 μl of MTT solution (5 mg/ml, Sigma). Finally, absorbance were measured at 550-560 nm.

Detection of cell cycle changes by flow cytometry

The cells as described above were removed from plates by trypsinization and pooled with cell culture supernatant containing nonadherent cells. Cells were washed once with PBS, fixed in cold 70% ethanol, and stored at -20 °C until analyzed. For staining, 1×106 cells were washed in PBS and stained in PBS with 50 μg/ml propidium iodide, 200 μg /ml boiled RNaseA, and 0.1% Triton X-100. Analyses were performed on a BD FACScan flow cytometer.

Transwell migration and invasion assays

The migration assay was performed using transwell cell culture chambers (8 μM pore size polycarbonate membrane, Costar). Briefly, cells were resuspended in RPMI-1640 with 0.1% FBS to a concentration of 3 × 105 cells/ml. The upper chamber was loaded with 100 μl of cell suspension and the lower chamber was loaded with 600 μl of RPMI-1640 with 10% FBS. After incubation for 24 h at 37 °C with 5% CO_2, the filter was fixed with 4% paraformaldehyde and stained with crystal violet (CV). The cells on the upper side of the filter were wiped off with a cotton swab. The cells migrated to the undersurface of the membrane were counted under a microscope. Ten microscopic fields (400×) were randomly selected to count cells. Each assay was done in triplicate.

The invasion assay was performed only one step different from the steps above. Before added cell suspension, the upper chamber was loaded with 100 μl matrigel mixture (matrigel: RPMI-1640=1:5).

Statistical Analysis

The results are presented as mean±SD from at least three independent experiments. (Statistical Package for the Social Sciences 14.0 statistical software). Comparisons between two groups were done using a Student's t test. A P-value of <0.05 was considered significant.

Results

The level of Aurora-A mRNA and protein in SGC-7901 were higher than in GES-1

The positive rate of Aurora-A mRNA was significantly higher in gastric cancer cell SGC-7901 than in normal gastric mucosa cell GES-1 (Figure 1a). It coincided with the level of protein (Figure 1b). So we chose SGC-7901 as an object of study. Our results coincided with the results in gastric cancer tissues.

Suppression of Aurora-A in SGC-7901 by siRNA or VX-680

In our study, it was found that SGC-7901 has higher level of Aurora-A mRNA than ones interfected using siRNA (Figure 2a). It coincided with the level of protein (Figure 2b). To verify the function of Aurora-A, we also

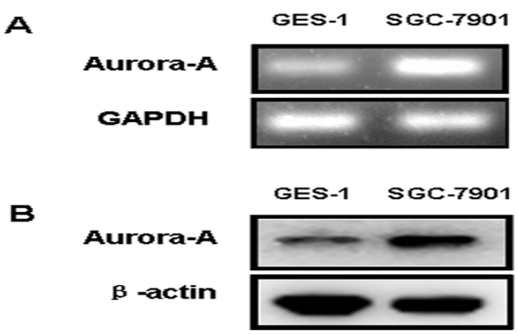

Figure 1. RT-PCR and Western Blotting Results. a) Detection of Aurora-A mRNA levels in GES-1 and SGC-7901. GAPDH was used as an internal control. b) Detection of Aurora-A protein levels in same set of cells by western-blot. β-actin used as an internal control.

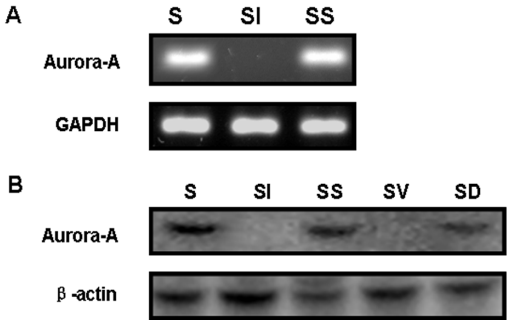

Figure 2. Interference Results. a) RT-PCR comparing treated with untreated and negative control. b) Aurora-A protein in SGC-7901, SGC-7901 treated with VX-680, SGC-7901 treated with DMSO, SGC-7901 interfered using siRNA and SGC-7901 interfered with scramble siRNA. (S: SGC-7901, SI: SGC-7901 interfered using siRNA, SS: SGC-7901 interfered with scramble siRNA, SV: SGC-7901 treated with VX-680, SD: SGC-7901 treated with DMSO)

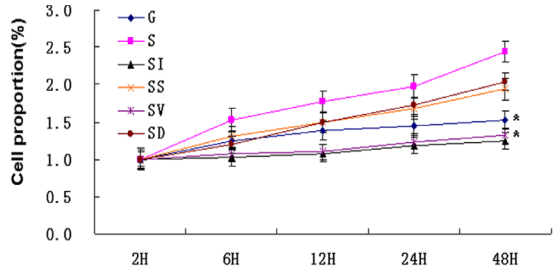

Figure 3. Growth Curve of Cell Lines Measured by MTT Assay. The experiments were conducted in triplicate and repeated three times (p,0.05)

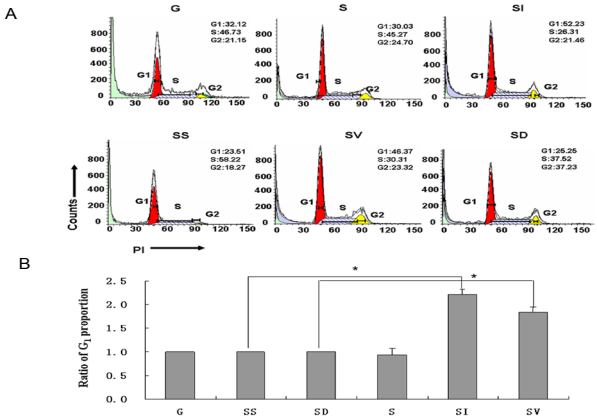

Figure 4. Growth Kinetics a) PI staining showed Aurora-A deletion caused G1 arrest. b) The histogram indicates significance.

utilized VX-680 a well-known Aurora-A inhibitor. After treated with VX-680 (100 nM, 48h), the level of Aurora-A protein showed lower than untreated ones (Figure 2b). However, there are no changes in negative control cells (Figure 2b).

The biological effects of Aurora-A deletion on SGC-7901

In order to further study the function of Aurora-A, we first utilized MTT to detect the viability of cells, including SGC-7901, SGC-7901 treated with VX-680, SGC-7901 treated with DMSO as control, SGC-7901 interfered using siRNA and negative control cells. The results showed deletion of Aurora-A may lead viability of cells to decrease (Figure 3) ($p<0.05$). The negative control cells showed no significant difference compared with untreated ones (Figure 3). We used PI staining to detect the effects of Aurora-A on cell cycle. As shown in Figure 4, the cells in G1 phase were increased in treated cells than untreated ones or negative control ones ($p<0.05$). Migration assay and invasion assay were carried out to measure the mobility of the cells. More untreated or negative control cells were found in the bottom membrane than deletion of Aurora-A ones (Figure 5) ($p<0.05$).

Discussion

In this study, we demonstrate that Aurora-A is crucial for development and cell cycle progression of gastric cancer cells. We utilized VX-680 and siRNA to induce dysfunction of Aurora-A. Previous studies have revealed that Aurora-A is overexpressed in gastric cancer tissues (Sen et al., 2002). We compared the level of Aurora-A mRNA and protein in gastric cancer cell SGC-7901 with normal gastric cell GES-1. The results coincided with previous studies and indicated that Aurora A is an oncogene in gastric cancer.

Wu et al (2005) has shown that overexpression of Aurora-A induces cell motility of MDCK cells. Consistent with our data, deletion of Aurora-A in gastric cell lines was inhibitory for proliferation. The results of Marumoto et al (2005) & Giet et al (2005) have shown that Aurora-A plays a series of important roles in the regulation of mitotic progression. It associated with various different cell cycle events, especially bipolar spindle assembly, centrosome maturation and separation. Aurora-A is crucial

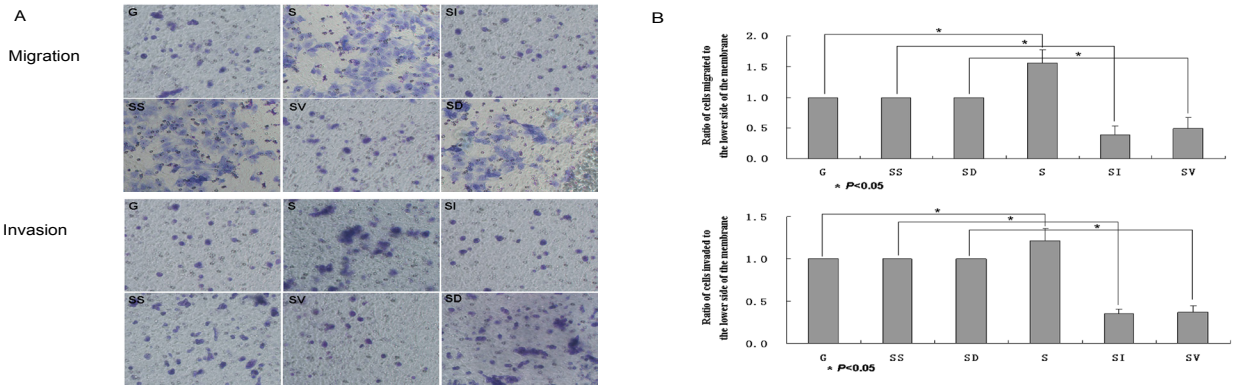

Figure 5. Transwell Assays. a) The mobility was measured by migration and invasion assay respectively. Upper picture showed migration assay and the lower one showed invasion assay. b) Quantitative results were obtained from three separate experiments. The significant difference between groups was statistically analyzed by SPSS 14.0.

for modulating cell cycle progression and maintaining genomic stability. Similarly, in our study, deletion of Aurora-A may induce cell G1 arrest. It demonstrated that Aurora-A can regulate cell cycle. It was reported to contribute indirectly to cdk1-cyclin B activation in Hela cells (Dutertre et al., 2003). However, whether Aurora-A can bind to some cell cycle proteins remains unclear. In the future studies, we will carry out CHIP and pull-down to find unknown proteins. Since migration and invasion of cancer cells contributed to metastasis of gastric cancer. We detected the mobility of the cells. Deletion of Aurora-A can suppress migration and invasion of gastric cancer cells.

VX-680 used in our study can bind to the ATP binding region of the Aurora kinase and lead it to dysfunction (Cheetham et al., 2007). Aurora-A inhibitors were exploited as potential cancer therapeutics. Indeed, some Aurora kinase inhibitors are currently in clinical trials (Keen et al., 2004; Carvajal et al., 2006). Although there is no significant side effect caused by short-term treatment, safety of long-term treatment is still a matter of debate (Yang et al., 2005; Warner et al., 2006). To our knowledge, we first detected the effects of VX-680 on gastric cancer cells. The results also provided a molecular basis for clinical treatment.

In summary, we first studied the effects of Aurora-A on gastric cancer cells.

Our studies showed clear evidence that deletion of Aurora-A may act as a suppressor in gastric cancer cells. All these evidence indicated that Aurora-A promoted pathogenesis and metastasis of gastric cancer cells and deletion of Aurora-A may be a potential therapeutic method to treat gastric cancer.

Acknowledgements

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