# Effect of Aurora A on Carcinogenesis in Human Prostate Cancer*

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**Abstract** **objective** To investigate whether Aurora A is involved in prostate cancer carcinogenesis by measuring Aurora A expression in prostate cancer. Methods A DNAzyme targeted to Aurora A was performed to detect the regulation of Aurora A in prostate cancer cell line PC3. **Results** We found that Aurora A was upregulated in 91% prostate cancer tissue and 3 prostate cancer cell lines. Suppression of Aurora A by DNAzyme arrests PC3 cell cycle and increases apoptosis. **Conclussion** Upregulation expression of Aurora A may be involved in prostate cancer carcinogenesis and Aurora A may be a valuable target for prostate cancer treatment .

**Key words** Aurora A; Gene expression; Prostate cancer; Carcinogenesis; DNAzyme

Prostate cancer is one of the most frequent malignant cancers, and is the second leading cause of cancer death among males in the Western word [1]. However, The molecular mechanisms for the carcinogenesis of prostate cancer are still poorly understood. A variety of chromosome aberrations, such as abnormal ploidy and improper segregation, are common mechanisms in cancer carcinogenesis[2]. Improper segregation can be caused by verity of factors, such as centrosome malfunction during mitosis[3]. Centrosome plays an important role in maintaining genomic stability by establishing bipolar spindles during cell division[4], and this action of centrosome is regulated by one of its associated protein, called Aurora A. Aurora A is localized to chromosome 20q13, a region frequently amplified in human malignant tumors[5]. Aurora A belongs to a family of kinases that are key regulators of centrosome duplication, chromosome segregation and cytokinesis [6], Therefore, Aurora is thought to be an important regulator involved in carcinogenesis.

Cells undergoing carcinogenesis possess two distinguishing characteristics[7]. One is a change in the amount or organization of DNA, the other is a loss of control of key mitotic checkpoints in DNA mitosis. Disruption of mitotic checkpoints in cell cycle can result in abnormal nuclei, missegregated chromosomes, and aneuploidy. Although the precise mechanisms by which that duplicate chromosomes are equally segregated during mitosis are largely unknown, the centrosome is believed to play an important role in the formation of equal segregation. During cell division, centrosomes form bipolar spindles, which ensure equal segregation of chromosomes to the two daughter cells. Therefore, abnormalities in centrosome duplication may result in an unequal segregation or a missegregation of chromosomes to the two daughter cells. Since Aurora A is one of the regulatory kinases of centrosome, which may participate in tumor formation and progression by regulating the function and stabilities of centrosome [8]. Recently, Aurora A has been proved to be involved in the carcinogenesis of many kinds of cancers [9]. However,

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whether Aurora A is expressed by and will plays a role in the carcinogenesis of prostate cancer remains unknown. To identify the expression and possible roles of Aurora−A in carcinogenesis of prostate cancer, we analyzed the expression of Aurora A in prostate cancer tissues and cell lines.

## *MATERIALS AND METHODS*

### Tissue samples

Twenty−four specimens of human prostate cancer which were confirmed pathologically were divided into two sections. One section was snap−frozen in liquid nitrogen and then stored in −80°C for reverse transcription−polymerase chain reaction (RT−PCR); the second section was paraffin embeded for immunohistochemical analysis. For the controls, 24 corresponding non−cancer prostate tissues (15 benign prostate hyperplasia (BPH) and 9 normal prostate tissues) were treated the same as above.

### RT–PCR

Total RNA was isolated from frozen tissue using TRZ REAGENT (SIGMA ST Louis, MO) according to the manufacturers instructions. RT−PCR was used to determine the expression of Aurora−A in 24 cancer tissues and 24 corresponding non−cancer prostate tissues. Briefly, cDNA was synthesized using reverse transcriptase (Omniscript, Qiagen). The PCR reaction was carried out using PCR Kit according to the manufactures protocol (Ampli−Taq Gold; Perkin Elmer, Wellesley, MA). The PCR reactions were performed as follows: 94 °C, 1 min; 58 °C,1 min; 70 °C, 90 s for 30 Cycles. Primer sequences were designed as follows: Aurora A (upstream): 5'−CATCATGGACCGATCTAA−3'; Aurora A (downstream): 5'−TGAACCGGCTTGTGACTG−3'. β−actin was used as an internal control. PCR products of Aurora A (219bp) and −actin (98bp) were resolved on 1.5% agrose gels and visualized with ethidium bromide.

### Immunohistochemistry

Paraffin−embedded tissue sections were dewaxed, rehydrated and blocked endogenous peroxidase and biotin, then antigen retrieval was performed. Sections were then incubated with the primary antibody overnight (Rabbit anti−BTAK/Aurora A antibody, Santa Cruz Biotechnology, CA, USA ) at 4 °C(1:150), followed by incubated with a 1:200 biotinylated rat anti−rabbit IgG (Santa Cruz Biotechnology, CA,USA ) at room temperature for 45 min. After an additional 60 min incubation with the ABC(Vector Laboratories), the sections were treated with 0.05% $H_2O_2$−3, 3'−diaminobenzidine at room temperature for 2 min. Each incubation was followed by three washes with TBS. After counterstaining with Mayer's hematoxylin, the sections were examined under a light microscope. A parallel negative control was performed using nonimmune serum instead of the primary antibody in each case.

### Cell culture and Western blot

Prostate cancer cell lines (PC3, LNCaP, Du145) were obtained from the American Type Culture Collection. The cell lines were routinely maintained in DMEM supplemented with penicillin/streptomycin and 10% fetal bovine serum. Aurora A mRNA expression in prostate cancer cell lines was detected by RT−PCR as described above. Western blot analysis was used to determine Aurora A protein expression in the three prostate cancer cell lines. Briefly, cell lysates were prepared by suspending cell pellets in lysis buffer Protein concentration was measured using DcProtein Assay Kit (Bio−Rad,Hercules,CA,USA). Equal amounts of protein (40μg )were separated on SDS−polyacrylamide gels by electrophoresis and then transferred on PVDF membranes (Amersham, Piscataway, NJ,USA).The membranes were blocked with 5% non−fat milk and then incubated with anti−BTAK/Aurora A antibody (1:500 Santa Cruz Biotechnology, CA, USA) for 1 h at room temperature. After washing with Tris−buffered saline with 0.03% Tween−20 (TBS−T), the membranes were incubated with 1:1500 horseradish peroxide−conjugated anti−rabbit IgG (Amersham, Piscataway, NJ,USA) and the signals were visualized by ECL detection system (Amersham, Piscataway, NJ, USA). Expression of actin was also measured as an internal loading control using an anti β−actin antibody (Santa Cruz Biotechnology).

**In vitro cleavage of Aurora A mRNA by DNAzymes**

**Cloning Aurora-A cDNA**

Homo sapiens partial coding sequence for aurora A was obtained from Genbank (accession No. AF011468). RT-PCR was used to clone the conserved fragment of Aurora A cDNA. First-strand cDNA synthesis was carried out from pooled RNA isolated from three prostate cancer cell lines described above and PCR was performed with the cDNA as template. PCR was initiated by denaturing at 95°C for 6 min, followed by 34 cycles (94 °C, 1 min; 58°C,1 min; 70°C, 90 s). Primer sequences were designed as follows: (upstream) with additional EcoRI site at 5'-GCGAATTC CATCATGGACCGATCTAA-3'; (downstream) with additional XhoI site at 5' -GCCTCGAGTTCAGGTGCCGATGGCAG -3'. The 341bp PCR product (325 bp of Aurora A cDNA and 16 bp of restriction endonuclease enzyme recognizing sites) was gel purified and ligated into the pc*DNA3.1*(+) plasmid between the *EcoRI* and *XhoI* sites. The new recombinant plasmid is called pc*DNA3-Aurora A*.

**Designing and synthesis of DNAzymes**

Three DNAzymes targeted to Aurora A mRNA were designed according to the specific rule of 10-21 DNAzyme [10] and synthesized by Roche company (Rhoche LTD,UAS). Each DNAzyme contains a catalytic domain of 15 highly conserved deoxyribonucleotides flanked by two substrate-recognition domains. The DNAzyme with this special structure can effectively cleave mRNA transcripts between unpaired purine and pyrimidine. Meanwhile, three parallel inactive DNAzymes with a single nucleotide substitution in the catalytic domain were synthesized as controls. The sequences of three DNAzymes targeted to Aurora A mRNA and the parallel controls are:

DNAzyme1:
5'-TTAGATCGGGCTAGCTACAACGACCATGATG-3'
Control1:
5'-TTAGATCGGGCTAACTACAACGACCATGATG-3'
DNAzyme 2:
5'-TTAACAGGGGCTAGCTACAACGACCTGAAAT-3'
Control 2:
5'-TTAACAGGGGCTAACTACAACGACCTGAAAT-3'
DNAzyme3:
5'-GCTTCTGAGGCTAGCTACAACGATCTGAACC-3'
Control 3:
5'-GCTTCTGAGGCTAACTACAACGATCTGAACC-3'

**Aurora A RNA in vitro cleavage assays**

pc*DNA3-Aurora A* was digested with *XhoI*. Aurora A mRNA template was synthesize using in vitro transcription kit of T7/SP6. Digoxin-labeled UTP was used to label Aurora A mRNA templates for in vitro transcription. The Dig-labeled Aurora A mRNA was 416 nucleotide (nt) including 325 nt of Aurora A RNA and 91nt of pcDNA3 sequence. Aurora A mRNA and DNAzymes as described above were mixed up at different molar ratios of mole in 20μl of reaction buffer containing 50mmol/L Tris-Hcl, pH7.2, 15 mM MgCl2, 0.01%SDS. After incubation at 40℃ for 2 hours, 96% formamide and 0.1% bromophenol blue in 20mmol/L EDTA were used to stop the reaction. The reaction RNA products were denatured with 50% formamide for 8 min, cooled down on ice, and then separated on 8% polyacrylamide gel made of 8 mol/L urea at the voltage of 500 v for 1.5 h. Gels containing RNA were transferred to nylon membranes. The RNA signals were tested by DIG -luminescence method according to the manufacturers instructions.

**DNAzyme transfection and cell cycle distribution in PC3**

From the pilot experiments of Aurora A RNA in vitro cleavage as described above, we found that only DNAzyme 2 could effectively cleave Aurora A mRNA in this study. We therefore use DNAzyme 2 in the following inhibition experiments in PC3 cells. After PC3 cells reached 60% confluent, DNAzyme 2 was transfected into PC3 cells using FuGENE 6 transfection reagent (Rhoche LTD,UAS.). To get efficient transfection, 4μg DNAzyme 2 was transfected into PC3 cells every 24 hr for 72 hr. Simultaneously, parallel control 2 was transfected into PC3 cells in different dishes. After the transfection, cells were collected to detect Aurora A protein expression at 12h,24h, and 48h following the final transfection. Meanwhile, the cells were analyzed by flow cytometry to determine the cell cycle distribution and cell apoptosis. Briefly, the cells

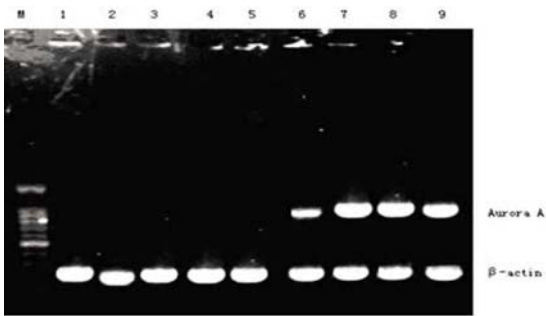


**Fig.1** Aurora A mRNA level in prostate tissues detected by RT-PCR

Lane1-3: normal prostate tissue; Lane 4-6: BPH; Lane7-9: Prostate cancer.

were collected and washed with PBS, then suspended in Krishan buffer (0.1% sodium citrate,0.2mg/ml RNAse, and 0.3%NP40) containing 0.05% propidium iodide and incubated at 4℃ for 30 min. The cells were subjected to counter flow cytometry.Data of cell cycle distribution were analyzed using ModFitLT2.0 (Verity Software House,Topsham,ME).

## *RESULTS*

### Aurora mRNA expression in prostate cancer tissues

To determine whether Aurora A is upregulated at transcriptional level, we analyzed Aurora A mRNA expression using RT-PCR in prostate cancer tissues. Among the 24 prostate cancer samples, Aurora A mRNA was upregulated in 22 cancer tissues (91%). However, in the 24 non-cancer prostate tissues, Aurora-A mRNA was expressed only in 1 of the 15 cases of BPH (6%) but none of the normal prostate tissues (Fig1).

### Aurora A protein expression in prostate cancer tissues

Since we found Aurora A mRNA was expressed in prostate cancer tissues, we next evaluate Aurora A protein expression and distribution in prostate cancer tissues using immunohistochemistry. We found that Aurora A protein localized to both cytoplasm and neucleus of the cancer cells. A strong cytoplasmic positive staining of Aurora-A was detected in 20 of the 24 (86%) prostate cancer samples. Only 1 case of BPH (6%) was moderately positive staining for Aurora-A, and there was no positive staining among the 9 normal prostate tissues (Fig.2).

### Aurora mRNA and protein expression in prostate cancer cell lines

To determine Aurora A expression in prostate cancer cell lines, we used RT-PCR to detect the mRNA expression and Western blot analysis to detect the protein expression in three prostate cancer cell lines PC3, LNCaP, Du145. We found that the expression of Aurora A was upregulated in all of the three of prostate cancer cell lines by RT-PCR (Fig.3 A, lane 1-3) and

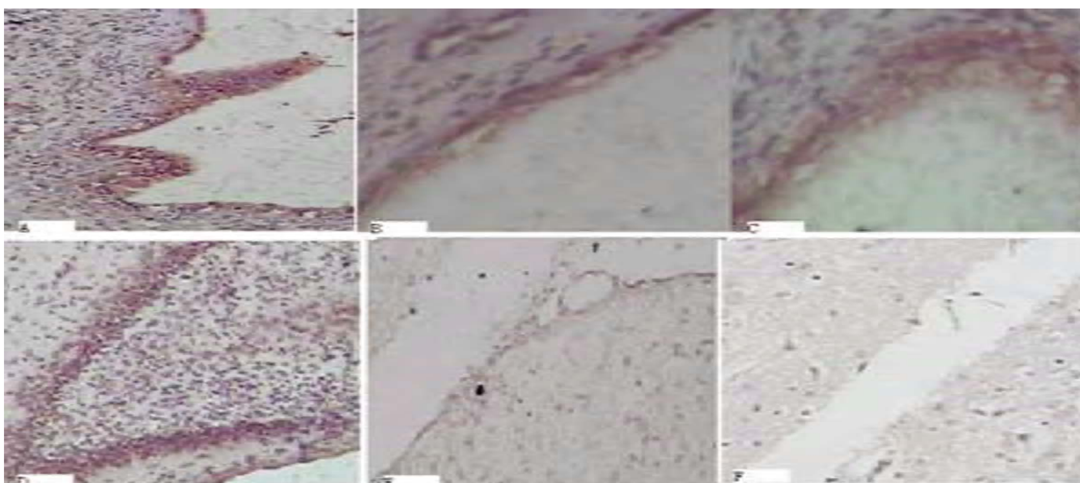

**Fig.2** Aurora A protein expression in prostate tissues detected by IHC(X400)

A-C:prostate cancer ; D-E:BPH; F:normal prostate tissue

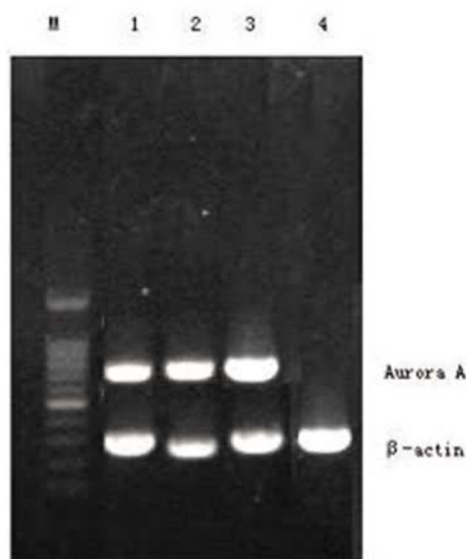

**Fig.3** Upregulation of aurora A in Pca cell lines
1.LNCap; 2.Du145; 3.PC3; 4 normal prostate tissue

**Fig.4** Effect of DNAzymes on the cleavage of Aurora A RNA
1. Aurora A RNA treated with inactive DNAzyme1(control 1)
2.Aurora A RNA treated with DNAzyme1
3. Aurora A RNA treated with inactive DNAzyme2(control 2)
4. Aurora A RNA treated with DNAzyme2
5. Aurora A RNA treated with inactive DNAzyme3(control 3)
6.Aurora A RNA treated with DNAzyme3

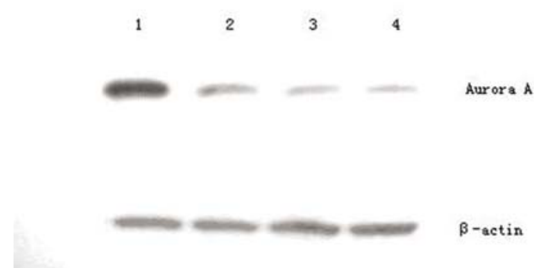


**Fig.5** Aurora A inhibition through DNAzyme 2 transfection in PC3 cells
Lane 1.control(inactive DNAzyme 2 treated); Lane 2. DNAzyme 2 treated(12h);
Lane 3. DNAzyme 2 treated(24h); Lane 4. DNAzyme 2 treated (48h)

Western blot analyses (Fig.3B, lane 1-3). However, there is no Aurora A mRNA (Fig.3A, lane 4) or protein expression (Fig.3B, lane 4) could be detected in normal prostate tissue.

## Cleavage of Aurora A mRNA by DNAzymes

DIG-labeled Aurora A mRNA was transcripted in and reacted separately with DNAzyme 1,2,3 for cleavage in vitro. The reaction products were separated through polyacrylamide gel electrophoresis and the signals were detected by DIG-luminescence method. We found that DNAzyme2 but not DNAzyme1 or 3 showed an efficient cleavage activity against Aurora A mRNA. After scanning using Gelwork 1D digital image analysis system (UVP), we found that DNAzyme2 could cleave about 64% of the Aurora A mRNA (Fig.4 lane 4). However, DNAzyme 1, DNAzyme3 and all of the three parallel controls could not cleave Aurora A mRNA (Fig.4).

## Inhibition of Aurora A inducing cell cycle arrest and apoptosis

Because DNAzyme2 could efficiently cleave Aurora A mRNA in this study, we next investigated whether DNAzyme2 could inhibit Aurora A protein expression

in PC3 cells using Western blot analysis. DNAzyme2 as well as control2 transduced PC3 cells were collected at 12h,24h,and 48h following the transfection.Anti−Aurora A antibody was used to detect its expression. We found that DNAzyme2 significantly inhibited Aurora A expression at 12h after transfection (Fig.5 lane2), with the inhibition of DNAzyme2 becoming more obvious at 24h and 48h (Fig.5 lane3 and 4) compared to the control. However, the control transduced PC3 cells did not show any difference in Aurora A expression between 12h,24h,and48h(data not shown). Furthermore, flow cytometry analysis showed that the inhibition of Aurora A through DNAzyme2 induced cell cycle arrest and cell apoptosis. We found that the percentage of cells in G2−M was 9.4% in control transduced PC 3 cells, with the percentage increasing to 13.3% at 12h, 20.8% at 24h and 29.2% at 48h after transfection (Fig.6). In addition, a fraction of cells in sub−G1 phase of the cell cycle was found at 48h in DNAzyme2 transduced cells, which represent for the apoptotic cells .(Fig.6D)

## *DISCUSSION*

Aurora kinases are highly conserved in eukaryotes and involved in many processes during cell division [11]. In this study, we found that the Aurora A was highly expressed in prostate cancer tissues and cell lines.

Three Aurora kinases have been identified in humans and designated as Aurora−A, −B, and C [12]. Aurora A protein is a centrosome−associated kinase, which functions in centrosome maturation and spindle assembly[6]. Previous studies demonstrated that aurora A was expressed in some kinds of cancers including breast cancer, colorectal cancer, ovarian cancer, hapatocellular carcinoma [13,14]. In this study, we found that the expression of Aurora A was upregulated in prostate cancer tissues and cell lines. Using RT−PCR and Western blotting, we first demonstrated Aurora A mRNA was over−expressed in 91% prostate cancer tissues but not in normal prostate tissues. A strong cytoplasmic Aurora−A protein expression was detected in 86% prostate cancer samples. The different positive percentage between mRNA and protein expression may due to the different sensitivity between RT−PCR and immunohistochemistry in detecting Aurora A expression. In addition, we found that both Aurora A mRNA and protein were upregulated in all the three

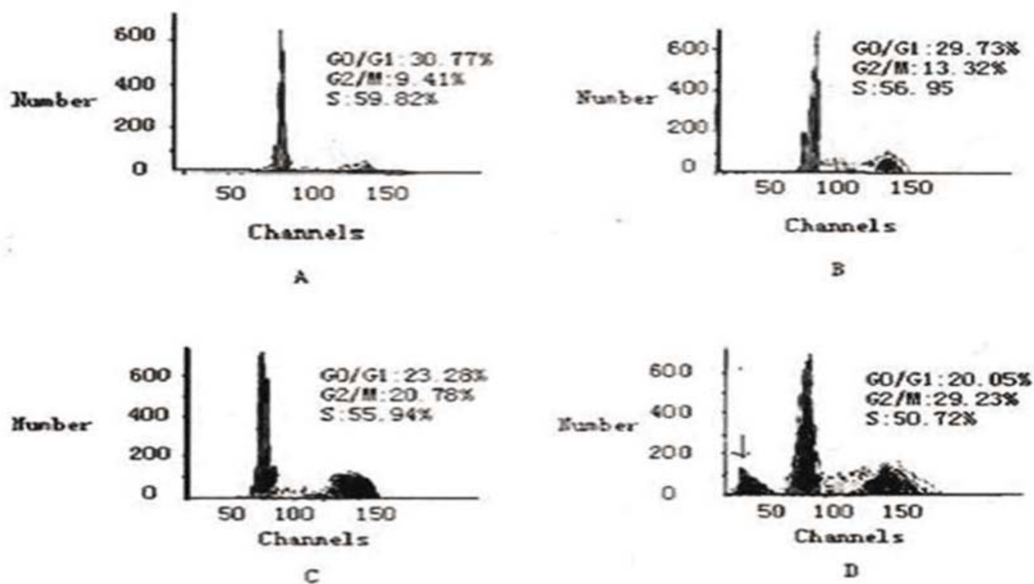


**Fig.6** Inhibiting aurora A arrests cell cycle and induces apoptosis

A.control(inactive DNAzyme 2 treated,48 hours); B.DNAzyme 2 treated (12h); C. DNAzyme2 treated (24h); D. DNAzyme2 treated(48h); Arrow indicate cells in sub−G1 phase.

prostate cancer cell lines in vitro, which is consistent with previous report that Aurora A is upregulated and might play a role in tumorigenic transformation of ovarian cancer cell[15]and gastric cell lines[16]. Therefore, our findings that Aurora A upregulation in prostate cancer tissues and cell lines suggest that Aurora A may be involved in the carcinogenesis of prostate cancer.

The mechanisms of aurora A causing carcinogenesis are not fully understood.The role of Aurora A in regulating mitosis is likely responsible for carcinogenesis [8].It has been shown that Aurora−A can phosphorylate several proteins that are important for mitosis. First, histone H3 which helps to converse the interphase chromatin to mitotic condensed chromosomes [17]; Second, cytoplasmic polyadenylation element−binding protein (CPEB )which promotes polyadenylation of cyclineB [18]; Third, TACC3, a protein essential for stablization and orgalization of microtubules [19]; Forth, Eg5,a kinesin−like protein involved in both centrosome separation and spindle assembly and stability[20]; Finally, TPX2 which is required to generate a stable bipolar spindle [21]. Through phosphorylation of these proteins functioning in cell mitosis, Aurora A plays important roles in genomic stabilities during cell cycle by regulating the dynamics of centrosome and microtubule. Therefore, when Aurora A was upregulated in cancer tissues, it will disturb the dynamics of centrosome and microtubule and thus drive genomic instability leading to carcinogenesis. Our findings that Aurora A was upregulated and might play a role in prostate carcinogenesis are in agreement with a recent study showing that Aurora A can upregulate oncogenes such as telomerase, c−myc and P53 and might contribute to oncogenesis[22].

We have also shown that suppression of Aurora A using DNAzymes arrests progression of the cell cycle in prostate cancer cell line (PC3). Furthermore, the DNAzmyes targeted Aurora A transdued PC3 cells undergo apoptosis. These findings suggest that Aurora A plays a role in promoting cell cycle, which results in cells overgrowing, since suppression of Aurora A arrests cell cycles and increase cell apoptosis in this study. Previous work demonstrated Aurora A appears to be largely and specifically up−regulated and amplified in cancer cells compared with normal cells[23]. Some studies showed Aurora A kinase was high in thymus, but had low levels in small bowel, testis, colon, spleen, and brain [24]. Another studies showed Aurora A to be elevated only in the testes [25]. This information suggests that an inhibitor targeting aurora A would have some tissue−specific selectivity, for tumor cells versus normal cells, thus causing low side effect.

In conclusion, we have shown that Aurora A is upregulated in prostate cancer tissues and cell lines, suppression Aurora A by DNAzyme arrests cell cycle and increases cell apoptosis in prostate cell line. Our findings suggest that Aurora A may play a role in prostate cancer carcinogensis, and inhibiting aurora A may be a valuable target for treatment of prostate cancer.

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