Evaluation of target mRNA cleavage by Aurorakinase B specific siRNA in prostate and hepatic cancer cells and its therapeutic potential in mouse models of liver cancer

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The anti proliferative potential of siRNA26, targeted to Aurora kinase B, in prostate cancer cells is known from a previous study from our laboratory. Here we first show that siRNA26 cleaves at the same position of the target mRNA in the prostate cancer and hepatocellular carcinoma cell lines, PC3 and HepG2 respectively. Aurorakinase B specific siRNA, but not a control siRNA, inhibited PC3 and HepG2 cell proliferation and cell migration. These effects correlated to RNA silencing of Aurorakinase B in both the cell lines. Intra-tumoral administration of HiPerfect complexed siRNA26 inhibited the growth of HepG2 xenografts in SCID mice. In an orthotopic setting, intravenous administration of HiPerfect encapsulated siRNA26 appeared to reduce the severity of multifocal lesions.

Keywords: Aurorakinase B, Hepatocellular carcinoma, siRNA, RNAi

Aurora kinase, an oncogenic serine/threonine (S/T) kinase family conserved across yeast, nematodes and mammalian cells, has been acknowledged to play an important regulatory function from G2 to cytokinesis, encompassing crucial cell-cycle events such as centrosome duplication, chromosome bi-orientation, and segregation. Inappropriate expression of Aurora kinases can induce aberrant mitosis, centrosome irregularities, and chromosomal instability, which lead to aneuploidy and cell transformation. Over-expression of Aurora kinase B (AURK-B), a key regulator of chromosomal and cytoskeletal events aiding correct segregation of genetic material into the dividing cells, has been reported in various human cancers.³⁻⁵ AURK-B directly phosphorylates histone H3 which contributes to chromosome number instability and mitotic chromosome condensation.⁶ Altered expression of Aurora kinases can lead to aneuploidy, increased chromosomal instability and cell transformation.⁷⁻⁸

Exploiting the RNA interference (RNAi) pathway is known as a potential therapeutic strategy against several cancers.⁹⁻¹¹ Limited evidence exists on the potential of AURK-B targeted RNAi as an anticancer therapeutic approach. A previous study from our laboratory showed that short interfering RNA (siRNA) mediated AURK-B knockdown inhibits proliferation of the human prostate cancer cell line PC3, but the potential of this approach to inhibit other cancers is not known. Besides, the above study did not specifically demonstrate RNAi mediated AURK-B mRNA cleavage although siRNA mediated degradation of AURK-B mRNA was demonstrated. Therefore the present study was taken up to examine RNAi mediated cleavage of AURK-B mRNA in prostate cancer and hepatic cancer cell lines, and to further evaluate the therapeutic potential of AURK-B specific RNAi against hepatocellular carcinoma (HCC).

Materials and Methods

Cell lines and siRNA—Cell lines PC3 and HepG2 were procured from American Type Culture Collection (ATCC), Manassas, VA, USA and cultured in a humidified atmosphere of 5% CO₂ at 37 °C in Eagle's Minimum Essential Medium supplemented with 10% Fetal Bovine Serum and Antibiotics. siRNA specific to human AURK-B (Genebank:NM_004217) designated

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siRNA26 or si26 [sense: 5’r(GGAGGAUC UACUAGAAUUCAGUAGAUCC UCUC)-3’, antisense: 5’r(U ACUCUAGAAUUCAGUAGAUCC CCUC)-3’], and two non-specific Control siRNAs, designated sic1 [sense: 5’r(GAGGAGGAAGCGCCCAA UAUC) dTdT3’, antisense: 5’r(GAUA UUGGGG GCUUCC UCCUC)dTdT3’] and sic2 [sense: 5’r(GAGAGUCAG GCCUUUUAG ACUGCdCdC3’, antisense: 5’r(GGGCAGUCUAAAAGGCCUGACU CUCUU)dTdT3’], were custom synthesized from Qiagen (Hilden, Germany). All transfections were carried out with 10 nM siRNA, using Hiperfect transfection reagent (Qiagen) as the transfection reagent. The transfection efficiency for each cell line was optimized to >90% using Cy3 or Cy5 labeled siRNA.

5′ RACE—Rapid Amplification of cDNA Ends was carried out using the 5'RACE kit version 2 (Invitrogen Product no. 18374-058). Briefly, cells were transfected with siRNA26 @10 nM per 2×10⁴ cells in 24 well tissue culture plates and total RNA was isolated at different time points using RNeasy kit (Qiagen). The RNA was converted into first strand cDNA using an AURK-B gene specific reverse primer 1 (5’CACGGGAGCGGGGAACTTTA3’), purified and poly C tailed as per the directions of the RACE kit manufacturer. The tailed first strand cDNA was PCR amplified using an abridged anchor forward primer (5′ GGGCAGUCUAAAAGGCCUGACUCUCUU)dTdT3’ specific to the Poly C tailed region at the 5′ end of the cDNA template and AURK-B gene specific reverse primer 2 (5’TGGCTTTATGTCTCTGTGAAT3’). The amplified RACE PCR products were visualized on a 1.5% agarose gel, desired fragments ligated into pGEMT-Easy plasmid (Promega) and the inserts sequenced using Big Dye Terminator Cycle sequencing Ready reaction kit v 3.1 RR-100 (Applied Biosystems), using a vector specific sequencing primer designated TPG18 (5′GTGT TCCCCATCACGAC3’). Since RNAi mediated cleavage of target mRNA is expected to be located between the nucleotides complementary to positions 10 and 11 of the siRNA antisense strand, the presence of such specifically cleaved AURK-B sequence following a Poly G abridged anchor primer sequence was considered confirmatory evidence for RNAi induction by siRNA26.

Western blot analysis—Total proteins prepared using MPER (Pierce), and quantified using Bradford’s reagent (Bio-Rad), were resolved on a 15% SDS PAGE and western blot analysis was carried out using a polyclonal antibody to AURK-B (Sigma). A monoclonal antibody (Sigma) was used to detect the internal control, α-tubulin. Goat anti-mouse IgG alkaline phosphatase conjugate (Sigma) was the secondary antibody used. For each treatment, densitometry was carried out for the AURK-B protein band using a Kodak imaging system and data analyzed using the Kodak MI software, and densitometric ratio (density of AURK-B band/ density of Tubulin, normalized with the values obtained for non-transfected control) was calculated.

qRTPCR analysis—From total RNA, first strand cDNA was prepared using Fast cell cDNA kit (Qiagen). Quantitative real-time Reverse Transcription Polymerase Chain Reaction (qRTPCR) was performed using the Applied Biosystems 7500 Realtime PCR system (Applied Biosystems) and data analyzed as per the instruction manual. β-actin was used as the internal control in all QPCR assays. Sequences of the primers and probes used were as follows. AURK-B: Forward primer 5’TTTCTAT GAGCTGCTGGTG3’, Reverse primer 5’CACAGAA GCCGGGGAACCTTTA3’, Probe 5′-6-FAM-ACACA ACGAGACCTATCGCC-MGB-3’, AURK-A: Forward primer 5’GTCACAAAGCG GTTCAGA AT3’, Reverse primer 5’AAAGCCCACT TGCCTCTTTTT3’, Probe 5′-6-FAM-CATCGGC CCTGAAAATAATCCCG-MGB-3’, β-actin: Forward primer 5’AGCCTCGCTTTGCCGA3’, Reverse Primer 5’CTGGTGGCTGGGCCGCG3’, Probe 5′-6-FAM-CGCCGGCGCTCCACACCAGGCC- MGB-3’. AURK-B and AURK-A mRNA levels was determined according to a widely accepted method. AURK-B mRNA levels in siRNA26 transfected cells, as a percentage of Non-transfected control cells, was shown as Percent mRNA levels. Percent AURK-B knockdown in the siRNA26 transfected cells was derived from the percent mRNA levels.

Cell proliferation and cell migration assay—Clonogenic (colony formation) assay was performed as follows. Briefly, 24 hours post transfection with siRNA26, cells were plated at a concentration of 200 cells per well in 6-well plates and incubated at 37 °C in a 5% CO₂ atmosphere. After 10 days of incubation, the resultant colonies were stained with 0.005% crystal violet and counted manually. Mean values of triplicate sets, with respect to non-transfected control, were obtained to determine the colony forming efficiency. Cell migration was evaluated by scratch assay on PC3 and HepG2 monolayers, according to a previously reported method.
Cell viability assay and IC₅₀ determination—Cell viability was determined using MTS reagent (Promega) according to the directions of the manufacturer. Cells were transfected with siRNA26 using five concentrations at one log increment each (0.01, 0.1, 1, 10, and 100 nM) in triplicates. 72 hours after transfection, floating and adherent cells were collected. The cells were stained with 0.4% trypan blue solution and viable and non-viable cells were counted using Neubauer chamber.

Cell cycle analysis—Cell cycle analysis was carried out based on previously published guidelines. Briefly, cells were transfected with equimolar concentrations of siRNA 26 or control siRNA. 72 h post transfection, cells were washed with PBS and fixed in 70% ice-cold ethanol at 4°C for 60 min. The cells, following a wash with PBS, were treated with Propidium Iodide (PI) for 30 min at 4°C. PI stained cells were subjected to flow cytometric analysis using FACS calibur, Becton Dickinson. Data were acquired and analyzed, for 20,000 gated events, using Cell quest software.

Generation of HCC tumor models in mice—All experiments involving animals were approved by the Committee for the Purpose of Supervision and Control of Experiments on animals, India. For xenograft tumor model generation, HepG2 cells were mixed with Matrigel (BD Biosciences) @ 5×10⁶ cells /0.1 mL. 4-6 week old female SCID mice (NIH strain) were injected subcutaneously on the flank and observed daily for tumor appearance. Tumors were measured in two dimensions (width × length) to assess growth using digital calipers (Mitutoyo cd-6 CSX). Tumor volumes were calculated [using the equation (axb²)/2, where a = largest diameter and b = smallest diameter, and expressed as mean ± standard deviation]. Orthotopic HCC model was generated by intrahepatic injection of HepG2 cells in SCID mice.

Experimental design and data analysis—All experiments were designed with a minimum of 3 replicates per group, unless otherwise stated specifically. Appropriate positive and negative controls were included unless otherwise stated. For in vivo experiments, age and sex matched animals were used in all groups and care was taken to collect data in an unbiased fashion. Comparison of the effects of various treatments was performed using Student’s t test. Quantitative results were expressed as Mean ± Standard Deviation and differences were considered significant at P ≤ 0.05.

Results and Discussion
5’RACE confirms RNAi mediated cleavage of AURK-B mRNA in siRNA26 treated HepG2 and PC3 cells—Judge et al. showed that 5’RACE analysis can be a suitable technique to establish RNA Induced Silencing Complex (RISC) mediated cleavage and degradation of the target mRNA by an exogenous siRNA in cells, as mentioned above. Activated RISC cleaves target mRNA precisely between the nucleotides complementary to positions 10 and 11 of the siRNA antisense strand, generating an mRNA cleavage product that is unique to the siRNA sequence, which can be detected by 5’RACE PCR. Therefore the cleavage site in the AURKB target mRNA is expected to be the underlined position in the sequence, 5’GAGGAGGATCTACTTGATTTCTAGA GTA3’. We extracted total RNA from non-transfected and siRNA26 transfected PC3 and HepG2 cells, and carried out 5’RACE. RACE PCR products corresponding to different time points after siRNA26 transfection, along with that from untreated control cells, were separated on 1.5% agarose gels. In the untreated control of both the cell lines, we observed a >600 bp RACE PCR product, corresponding to the expected amplicon size from an uncleaved AURK-B mRNA template. In contrast, a smaller PCR fragment, approximately ~150 bp in size, was also observed at all time points since 24 h post transfection with siRNA26 in PC3 cells as well as HepG2 cells. The RACE PCR products corresponding to 30 h after transfection for both the cell lines, along with the respective untransfected controls, are shown. In both cell lines transfected with siRNA26, the appearance of this ~150 bp band coincided with the disappearance of the band corresponding to the uncleaved AURK-B template (Fig. 1a). Since 152 bp corresponds to the expected amplicon from an siRNA26 cleaved AURK-B mRNA template, as explained above, this result suggested that siRNA26 induces RNAi mediated cleavage of AURK-B mRNA in both the cell lines. In order to confirm this, the 152 bp RACE PCR products from siRNA 26 treated PC3 and HepG2 cells, corresponding to 24 hours and 30 hrs (post transfection) respectively, were ligated into pGEMT-Easy pasmid (Promega) by TA cloning, and sequenced. Results revealed the presence of poly G abridged anchor primer sequence precisely at the expected RNAi cleavage site between the A and T at positions 10 and 11 from the 3’ end of the siRNA26 target site in the RACE products derived from siRNA26 treated PC3 (Fig. 1b) as well as HepG2 cells.
These results confirmed that siRNA 26 induces RNAi in prostate cancer as well as HCC cells leading to RISC mediated cleavage of AURK-B mRNA as expected.

**AURK-B RNA silencing inhibits proliferation of PC3 and HepG2 cells**—The anti-proliferative effect of AURK-B RNA silencing in PC3 cells is known\(^\text{12}\), as mentioned above. Having confirmed RNAi mediated AURK-B mRNA cleavage by siRNA26 with comparable efficiency in PC3 as well as HepG2 cells, we evaluated the effect of siRNA26 on proliferation and migration of both the cell lines by clonogenic assay and scratch assay respectively. In this experiment we included a control siRNA transfected group, in addition to the non-transfected control group. Transfection with siRNA26, but not equimolar concentration of control siRNA, resulted in comparable knockdown of AURK-B protein (Fig. 2a) and mRNA levels (Fig. 2b and Supplementary data Fig. S1) in both PC3 and HepG2 cells. Parallelly we evaluated the effect of AURK-B knockdown on cell proliferation and cell migration by

**Fig. 1**—Visualization of 5’RACE PCR products from siRNA26 transfected cells. HepG2 and PC3 cells were transfected with siRNA26. Total RNA was extracted and 5’RACE was performed as described under Materials and Methods. (a) Agarose gel electrophoresis of RACE PCR products from Non-transfected (NT) and siRNA26 transfected (si26) cells is shown. The RACE PCR bands corresponding to uncleaved and RNAi-cleaved AURK-B mRNA are indicated. M1, M2: DNA markers. (b and c) The ~152 bp RACE PCR products from siRNA26 transfected cells were ligated in pGEMT-Easy vector and the insert sequenced using a vector specific primer. Chromatograms revealing the presence of the poly G anchored primer sequence followed by the RNAi cleaved sequence in the AURK-B mRNA template are shown: (b) PC3 (c) HepG2.

**Fig. 2**—siRNA26 transfection inhibits AURK-B expression in PC3 and HepG2 cells. (a) PC3 and HepG2 cells were transfected with siRNA26 (si26) or equimolar control siRNA (siC2) or left non-transfected. Inhibition of AURK-B protein expression in siRNA26 transfected HepG2 cells, at 72 hours post transfection, is shown by western blot. Tubulin was used as internal control in the western blot. AURK-B knockdown was quantitated by densitometry of the blot. Densitometric ratios are shown as inset. (b) PC3 and HepG2 cells were transfected with siRNA26 (si26) or equimolar control siRNA (siC1) or left non-transfected. AURK-B RNA silencing was evaluated by qRTPCR and percent AURK-B knock down in siRNA26 (si26) transfected cells over the respective control siRNA transfected cells, at 72 hours post transfection, is shown.
clonogenic assay and scratch assay respectively. Results showed specific and significant inhibition of colony formation in both the cell lines as a result of AURK-B knockdown (Fig. 3a), confirming that AURK-B plays a role in the proliferative potential of both the cell lines. Results of scratch assay showed that cell migration is inhibited in siRNA26 transfected cells compared to Control siRNA transfected PC3 (Fig. 3b) and HepG2 (Fig. 3c) cells. But the inhibitory effect on cell migration was more prominent in PC3 cells than HepG2 cells. One reason for this discrepancy could be that PC3 cells grow as monolayers while HepG2 tends to grow in clusters. However, the above results suggested that AURK-B RNA silencing has broad spectrum anti-proliferative potential against prostate cancer and hepatic cancer cells. Further studies were focused on siRNA26 mediated inhibition of hepatic cancer.

**AURK-B knockdown reduces viability and survival of HepG2 cells—** Next the effect of siRNA26 transfection were tested on HepG2 cell viability and cell membrane integrity by MTS assay and Trypan Blue exclusion assay respectively. Consistent with the above results on cell proliferation, cell viability was found to be significantly reduced following transfection of the cells with siRNA26 compared to Non-transfected and Control siRNA transfected cells (Fig. 4a). A dose
dependent reduction was also observed in cell membrane integrity after transfection with siRNA26, but not with equimolar Control siRNA. It was assumed that all cells with disrupted cell membrane integrity have entered the death path way, and thus the half maximal inhibitory concentration (IC\textsubscript{50}) of siRNA26 in these cells was found to be 0.6 nM (Fig. 4b). The difference in percent viability (MTS assay) and percent survival (Trypan blue exclusion assay) may be due to the different working principles for the two assays. Trypan blue exclusion assay is based on the principle that live cells that have intact cell membranes exclude the dye. On the other hand, MTS assay is based on the principle of reducing the tetrazolium dye MTS to its insoluble form, which has a color. Thus MTS assay will detect all metabolically viable cells, even though the cell membrane integrity of a metabolically viable cell may be disrupted.

Cell cycle analysis, by flow cytometry, revealed a significant increase in the population of cells in the sub G1 phase and a corresponding reduction in the population of cells in the G0-G1 phase, specifically in response to siRNA26 transfection. A modest, but significant increase in the population of cells corresponding to polyploidy was also noticeable (Table 1). The flow cytometry histogram profiles corresponding to the different treatments are provided as supplementary data (Fig. S2). Taken together, the above results showed that siRNA26 mediated AURK-B knockdown significantly reduces cell viability and cell cycle progression in hepatic cancer cells, and further confirmed that AURK-B RNA silencing may have therapeutic potential against HCC. The modest increase in the population of cells corresponding to polyploidy was consistent with the observation of Warner et al.\textsuperscript{28}, who evaluated Aurora kinases A and B as molecular targets for anti cancer therapy, using antisense oligonucleotides.

**siRNA26 inhibits growth of HepG2 xenografts in mice**—Next we evaluated the in vivo therapeutic potential of siRNA26 against HCC. First of these evaluations was done by intra-tumoral administration of the siRNA in a xenograft model in SCID mice. HepG2 xenograft tumors were induced in SCID mice, and when the tumors attained a volume of 30–40 mm\textsuperscript{3}, they were randomized into three groups viz., untreated control (n=3), treated with HiPerfect diluted in PBS (Placebo; n=4), and treated with HiPerfect encapsulated siRNA26 (n=5). For all treated groups, 2 intratumoral injections of 25 µg siRNA26 each, or an equal volume of placebo, were given every week for 4 weeks. During the course of the treatment, the animals were observed everyday for clinical signs and tumor volumes were measured every third day. Significant inhibition of tumor growth was observed in the group of animals that received siRNA 26 compared to the group that received placebo. The difference in tumor volumes (mm\textsuperscript{3}) between siRNA26 treated and placebo treated groups was statistically significant at 0.5 week (p = 0.00977), 1.5 weeks (P = 0.00024), 3 weeks (P = 0.00062) and 4 weeks (P = 0.00147) after commencement of treatment (Fig. 5a). The tumor of the animal that showed the best therapeutic response at the end of the 4\textsuperscript{th} week of treatment, shown along side representative control tumors from untreated and placebo treated mice, (Fig. 5a inset) was evaluated for inhibition of AURK-B mRNA and protein levels. Compared to the AURKB mRNA level in the untreated control tumor, the siRNA26 treated tumor showed ~62% reduction in AURK-B mRNA level, but the placebo treated tumor showed ~100% increase in AURK-B mRNA levels (Fig. 5b). Body weight changes were not monitored in this experiment. Nevertheless, these results confirmed that siRNA mediated AURK-B knockdown can be a viable approach to inhibit progression of hepatic cancer in vivo.

### Table 1—Percentage of cells in different stages of cell cycle, following siRNA26 transfection

<table>
<thead>
<tr>
<th>Phase</th>
<th>Percentage of cells</th>
<th>P value (siC vs si26)</th>
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<tbody>
<tr>
<td></td>
<td>Untreated</td>
<td>HiPerfect</td>
</tr>
<tr>
<td>G0-G1</td>
<td>47.98 ± 0.61</td>
<td>49.91 ± 0.78</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>10.21 ±1.32</td>
<td>10.45 ± 0.9</td>
</tr>
<tr>
<td>Polyploidy</td>
<td>1.89 ± 0.11</td>
<td>1.58 ± 0.07</td>
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<tr>
<td>G2-M</td>
<td>12.20±0.48</td>
<td>14.27±2.57</td>
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<tr>
<td>S</td>
<td>15.46 ± 0.25</td>
<td>13.84 ± 2.78</td>
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* HepG2 cells were transfected with siRNA26, equimolar control siRNA1, HiPerfect alone or left untransfected. At 72 h post transfection, cell cycle analysis was performed. Percentage of cells in different stages of cell cycle, at 72 h post transfection.
Intra-venous administration of HiPerfect encapsulated siRNA26 modestly reduces the severity of multifocal lesions in an orthotopic model of HCC—In a separate study we evaluated the therapeutic potential of intravenously administered siRNA26 in an orthotopic HCC model generated by intra hepatic injection of HepG2 cells, as mentioned under Materials and Methods. Within 7 days of injecting the cells, the liver was found to be highly metastasized. HiPerfect encapsulated siRNA26 was then administered @ 200 µg per injection, once in 2 days for 10 days, starting from day 7 post injection of cells into the liver (n = 5). Tumor induced animals that were not treated with siRNA26 (n =5) and a normal animal, not induced for tumor development, were included in the experiment. On days 1, 5, 7, 9 and 12 after the last dose of siRNA26 / placebo, a representative animal from the HCC induced-siRNA26 treated group and HCC induced-untreated control group were sacrificed to harvest the liver for visual comparison of disease severity. On days 1, 9 and 12, no appreciable amelioration of intra-hepatic tumor metastasis was noticed in response to siRNA26 treatment, with the representative liver specimens from the two groups being similar in appearance (Fig. 6A). However, histopathological examination of the liver tissues harvested on day 12 after the last dose of siRNA26, by H&E staining, revealed a reduction in the severity of multifocal lesions and inflammatory infiltrates in the liver of the HCC-induced siRNA26 treated animal compared to the liver of the HCC-induced untreated control animal (Fig. 6B). Thus the therapeutic potential of siRNA26 against the orthotopic HCC model - under the conditions used in this study—appeared to be limited, unlike that observed against the xenograft HCC model.

RNAi technology has been effectively used to silence various proteins intractable to inhibition by conventional methods, opening up novel therapeutic possibilities. However siRNA therapeutics will have to overcome certain key barriers, especially in drug delivery systems. It is also equally important to identify and validate highly potent targets for siRNA therapeutics. Regulatory kinases have traditionally been attractive targets for anti cancer therapeutics in general. Aurora kinases are one such family of
kinases, with majority of studies reported using conventional inhibitors. Although early studies on Aurora kinases, as anticancer therapeutic targets, centered around AURK-A, recent studies\textsuperscript{12,21-26} have recognized AURK-B as a novel anti cancer target. Of these, a study from our laboratory, by Addepalli et al\textsuperscript{12} demonstrated the inhibitory potential of AURK-B specific siRNA against proliferation of prostate cancer cells. However that study did not show siRNA mediated AURKB mRNA cleavage. In the present study we first demonstrate that the above siRNA cleaves AURK-B mRNA at the same location in the prostate cancer cell line PC3, as well as the HCC cell line HepG2. With a low IC\textsubscript{50}, the siRNA was highly potent in significantly reducing AURK-B mRNA levels as well as protein expression in Hepatocellular carcinoma (HCC) cells, and was capable of inducing apoptosis in them. In this context, our observations were consistent with Marxer et al\textsuperscript{27} who reported that AURK-B inhibition induces apoptosis in Hep3B cells. Our results are also consistent with Warner et al.\textsuperscript{28}, who discussed the potential of AURK-A and AURK-B as potential anti cancer targets.

HCC is one of the most common malignancies worldwide, which can be induced by prolonged exposure to Hepatitis B or C virus (HBV, HCV) infections or toxic reagents such as aflatoxin B1. Resection, transplantation or percutaneous and transarterial interventions, conventional chemotherapy and radiotherapy are of limited efficacy in advanced stages of this rapidly fatal disease. Accumulation of multiple genetic and epigenetic alterations can play an important role in the progression of HCC\textsuperscript{27-30}. With molecular mechanisms of hepato-carcinogenesis still being understood, there is an urgent need to identify optimal targets for the treatment of HCC\textsuperscript{31-34}. Proof of concept studies on RNAi based therapeutic approaches against HCC have been reported\textsuperscript{35,36}. In another study\textsuperscript{36}, aberrant expression of AURK-B and phosphorylated H3 were linked to hepatocarcinogenesis. In the present study, we show that RNA silencing of AURK-B can be a potential therapeutic strategy against HCC, in addition to validating target mRNA cleavage by siRNA26 in prostate and hepatic cancer cells. Our results are also consistent with earlier studies using AURK-B inhibitors\textsuperscript{37}.

In conclusion, the significance of the present study is in the context of novel target discovery for broad spectrum anti-cancer RNAi therapeutics. The data obtained from \textit{in vitro} experiments and \textit{in vivo} experiments using a xenograft mouse model demonstrate the broad spectrum anti cancer potential of AURK-B RNA silencing. However, we acknowledge that intravenous administration of siRNA26 in an orthotopic mouse model of HCC in the present study only showed a limited therapeutic response. Further studies will be required, in an orthotopic setting, to optimize targeted \textit{in vivo} delivery strategies to accomplish the best therapeutic efficacy. Particularly, detailed studies will be necessary to further optimize the induction of orthotopic HCC model, as well as the therapeutic regimen of AURK-B targeted siRNA in this model.

**Supplementary data**

Supplementary data (Figures S1 and S2) associated with this article may be obtained from the correspondent author on request.

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**References**


