**Molecular and Cellular Pathobiology**

**Aurora A Is a Repressed Effector Target of the Chromatin Remodeling Protein INI1/hSNF5 Required for Rhabdoid Tumor Cell Survival**

SeungJae Lee1, Velasco Cimica1, Nandini Ramachandra1, David Zagzag2, and Ganjam V. Kalpana1

**Abstract**

Rhabdoid tumors (RT) are aggressive pediatric malignancies with poor prognosis. INI1/hSNF5 is a component of the chromatin remodeling SWI/SNF complex and a tumor suppressor deleted in RT. Previous microarray studies indicated that reintroduction of INI1/hSNF5 into RT cells leads to repression of a high degree of mitotic genes including Aurora Kinase A (Aurora A, STK6). Here, we found that INI1/SNF5 represses Aurora A transcription in a cell-type–specific manner. INI1-mediated repression was observed in RT and normal cells but not in non-RT cell lines. Chromatin immunoprecipitation (ChIP) assay indicated that INI1/hSNF5 associates with Aurora A promoter in RT and normal cells but not in non-RT cells. Real-time PCR and immunohistochemical analyses of primary human and mouse RTs harboring mutations in INI1/hSNF5 gene indicated that Aurora A was overexpressed/derepressed in these tumor cells, confirming that INI1/hSNF5 represses Aurora A in vivo. Knockdown of Aurora A impaired cell growth, induced mitotic arrest and aberrant nuclear division leading to decreased survival, and increased cell death and caspase 3/7-mediated apoptosis in RT cells (but not in normal cells). These results indicated that Aurora A is a direct downstream target of INI1/hSNF5-mediated repression in RT cells and that loss of INI1/hSNF5 leads to aberrant overexpression of Aurora A in these tumors, which is required for their survival. We propose that a high degree of Aurora A expression may play a role in aggressive behavior of RTs and that targeting expression or activity of this gene is a novel therapeutic strategy for these tumors. *Cancer Res;* **71**(9); **3225**–**35. © 2011 AACR.

**Introduction**

Rhabdoid tumors (RT) of the kidney (malignant rhabdoid tumors or MBT), brain (atypical teratoid and rhabdoid tumors or AT/RT), and soft tissues (extrarenal rhabdoid tumors or ERT), are rare, aggressive, and incurable pediatric malignancies. There are no effective or standard treatment strategies for these tumors or MRT, are rare, aggressive, and incurable pediatric malignancies. There are no effective or standard treatment strategies for these tumors.

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Many cancer cells have a weakened mitotic check-point because of overexpression of genes necessary for mitotic progression and the inhibitors of mitotic genes are effective anticancer agents (17). Aurora A is frequently overexpressed in many cancers, including breast, lung, pancreas, liver, and head/neck (18, 19); and overexpression of Aurora A has been implicated to lead to genetic instability and tumorigenesis (19, 20). Aurora A expression is indicative of poor prognosis in many cancers (18, 21, 22). Aurora A is a member of the family of genes including Aurora A, B, and C, all of which are serine/threonine kinases that play multiple roles in mitosis. Expression of Aurora A is regulated during cell cycle and it peaks during G2/M phase (23). Aurora A is also involved in phosphorylation of several substrates including PLK1 to promote entry into mitosis at the G2/M phase (24–26). Aurora A is an attractive target for developing anticancer therapy because of its association with poor prognosis and because of its kinase activity. Currently, many Aurora A kinase inhibitors are under phase I clinical trials (27–30).

Based on our microarray studies that Aurora is repressed by INI1 in MON cells, we hypothesized this gene could be derepressed in RTs that arise because of loss of INI1/hSNF5 and that this gene could be a potential drug-target to inhibit RT growth. To test this hypothesis, we first carried out transcription and chromatin immunoprecipitation (ChIP) assays to determine if Aurora A is a direct downstream effector of INI1/hSNF5 in RT cell lines. We tested the possibility that Aurora A is derepressed/overexpressed in RTs independent of the stage of cell cycle. Furthermore, we carried out studies to determine if downmodulation of Aurora A is effective in inhibiting RT cell survival. Our study indicates, for the first time, that INI1 represses Aurora A in RT cells, that it is highly expressed in human and mouse RTs and that downmodulation of Aurora A potently inhibits RT cell growth. These observations provide a strong basis for therapeutic interventions of RTs by targeting Aurora A gene expression or activity in the clinic.

Materials and Methods

Cell lines

RT cells [MON, derived from Abdominal wall MRT, a gift of Dr. Dellattre, Institute Curie, Paris; STA-WT1, derived from kidney MRT, a gift of Dr. Ambros, Children’s Cancer Research Institute, Austria; A204 derived from muscle RT [American Type Culture Collection (ATCC) #HTB-82]; and G401, derived from kidney MRT (ATCC #CRL-1441)] were cultured in RPMI supplemented with 10% FBS. Non-RT cell lines including HeLa (Sigma #HS3021013), 293T (ATCC #CRL-11268), Glioma, and SF268 (a gift of Dr. Agu, Albert Einstein College of Medicine) were cultured in DMEM supplemented with 10% FBS. Jurkat and CEMX-174 (NIH AIDS research reagents and repository) were cultured in RPMI supplemented with 10% FBS. Normal diploid fibroblasts, CRL-2522 (ATCC #CRL-2522) and BJ-TERT (kind gift Dr. J. Greally, AECOM), were cultured in MEMα + Glutamax and DMEM respectively, supplemented with 10% FBS.

Plasmids and expression vectors

INI1/hSNF5 was reintroduced into RT cells using pCGN-INI1 (expressing hemagglutinin tagged full length INI1/hSNF5) and pBabe-puro-INI1 (retroviral vector expressing flag-tagged full length INI1/hSNF5. Flag-INI1/ hSNF5: ref 31). Aurora A reporter plasmids expressing luciferase gene fused to Aurora A promoter regions of various length starting from −1,486, −415, −124, or −75 to +354 (pGL-Aurora-A-Luc) were utilized for transient transcription assays (23). The pRL-SV40 Renilla luciferase plasmid was used as an internal normalization control. A lentiviral vector expressing INI1-shRNA was constructed as follows (32). Briefly, 2 oligonucleotides corresponding to INI1 shRNA (forward: gatccccgacgatcttgattagatgctgg actttttgaaa and reverse: agctttccaaaagtgacgatctgg atttgaatctcttgaattc aatc cagtagctgaccgg) were annealed and subcloned into the BglII and HindIII site of pSUPER vector. A BamHI–SalI fragment of pSUPER-shINI1 was subcloned into the BamHI–SalI site of pRD1292 (gift of Dr. Trono D. University of Geneva, Switzerland) to obtain pRDI-shINI1.

Transfection and transcription assay

RT cells were either (i) cotransfected with plasmid expressing HA-INI1 along with 1/10 amounts of pEGFP using Dharmfect 1 (Dharmacon, #T2001-02), selected with genetin (250 ug/mL) for 6 to 7 days post transfection; or (ii) transfection with pBabe-puro-INI1, selected for puromycin for 3 days, followed by RNA and protein extraction. For transient transcription assay, HeLa or MON cells were cotransfected with pGLaurora A-Luc along with vector expressing HA-INI1 or control vector. Cells were harvested 5 days posttransfection and assayed for luciferase activity using the Dual-Glo luciferase assay system (Promega, #E1910) with Renilla activity measured as an internal control. Generation and infection of retroviral/lentiviral vectors expressing Flag, Flag-INI1, and shAurora A were carried out as previously described (14).

RT-PCR analysis

Total RNA was isolated from transduced or transfected MON cells using RNeasy kit (Qiagen, #74104). Reverse transcription-PCR (RT-PCR) was carried out as previously described (14) using primers to amplify cDNAs of Aurora A [forward: 5’-ggagagcttaaaattgcagatttg-3’, reverse: 5’-aggtctcttgtagtttgcc-3’ (33)] and glyceraldehyde-3-phosphate dehydrogenase (GAPDH: 14). Information on quantitative real-time PCR analysis using human and mouse RTs and cells, and normal cell lines and tissues, is provided in the Supplementary Material section.

ChIP

ChIP analysis was conducted as described (34, 35) with minor modifications. Details of this procedure are provided in the supplement.

Immunoblot analysis

Western blot analysis was carried out as previously described (14) using the following antibodies: α-FLAG M2-peroxidase (SIGMA, #AF8092), α-HA-peroxidase (SIGMA,
#H6533), α-PLK1 (Upstate, #06-831) α-Aurora A kinase (BD Transduction Laboratories, #610938), α-Tubulin (SIGMA, #5168) and α-glyceraldehydes-3-phosphate dehydrogenase (GAPDH; CHEMICON, #MAB374).

Immunohistochemical analysis

Staining was conducted on paraffin sections of the following tumor samples and normal tissues: (i) 3 human AT/RTs (thn09-5079, s99-16511, and s06-2106) and a human normal brain tissue adjacent to a tumor as a control; (ii) 2 mouse primary tumors spontaneously developed in Ini1+/− mice, HCP04-587E (soft tissue face tumor) and HCP05-993#2 (brain tumor); and (iii) 1-xenografted tumor (HCP0-597-A1xB) derived from human G401 RT cells developed in the flanks [by subcutaneous injection of the cells (36)]. For staining INI1, α-IN1I antibodies (BD Biosciences Cat# 612110) were used. The immunohistochernistry was conducted essentially as previously described with minor modifications (37). Details are provided in the Supplementary Data.

RNA interference analysis

Small interfering RNAs (siRNA) to PLK1 (38), Aurora A [AUGCCUCUGCUACUGUCA (39)], and INI1/hSNF5 (SMARCB1, GUGACGACUGGGAAUGGAAUU) with an additional 2 thymidine residues (dTdT) at the 3′ end were purchased from Dharmacon. Cy3-luciferase GL2 Duplex (Dharmacon, #D-001110-01-05) was used as a control. A lentivector, pLKO.1, expressing scrambled shRNA was purchased from Addgene. Additional 2 shRNA (in lentivectoral constructs) were used to downmodulate Aurora A.

shAurora A 5′...ccggacgaaagtgtctacttatactcagatata-agttgacaattctcgttt...5′

shAurora A-1 5′...ccggacgacaattctcagatcagctgt-agcttaaacaggtcctgtttt...5′

from SIGMA (#H6533) with TRC #s of N0000000655 and N0000199288, respectively. Lentivectoral vectors expressing scrambled, INI1/hSNF5 or 2 different Aurora A shRNA were used for transduction and the cells were selected for 2 to 3 days with puromycin (1 ug/mL) before harvesting.

Immunofluorescence, cell cycle, and caspase-3/7 assays

Immunofluorescence, cell cycle, and caspase assays were carried out essentially as previously described (14).

Cell proliferation assay

Cell proliferation of RTs and normal cells were carried out using CellTiter 96 Aqueous One Solution Assay (Promega, #G3811). The quantity of formazan product of the viable cells was measured by the amount of 490 nm absorbance using a plate reader (Wallac, 1420 multilabel counter). After 2 days of selection with puromycin, the same number of shRNA transduced cells (5,000 and 25,000 cells) was plated in 96-well plates in triplicate. Every 24 hours, the medium was replaced with 120 μL of diluted assay solution and incubated for 1 hour and absorbance was measured.

Background absorbance (media alone) was subtracted from that of the samples.

Results

Cell-type–specific repression of Aurora A by INI1/hSNF5

Microarray analysis indicated that reexpression of INI1/hSNF5 in MON cells represses a large number of mitotic genes including Aurora A (14). However, it was unclear if Aurora A is a specific target of INI1/hSNF5 in all the RT cells and if INI1/hSNF5 has a direct role in repressing this gene. Since Aurora A could provide clues to poor prognosis of RTs and since it could serve as a potential drug target, we further investigated the repression of Aurora A by INI1/hSNF5 in various RT cells. To confirm the microarray results, first, MON cells were transduced with retroviral vectors expressing Flag-INI1 or Flag (empty vector) and selected with puromycin and tested for expression of Aurora A by RTs both at the RNA and protein levels. Reintroduction of INI1/hSNF5 led to the repression of Aurora A at both mRNA and protein levels (Fig. 1A and B). These results indicated that INI1 represses transcription of Aurora A, confirming the microarray results. Furthermore, reintroduction of INI1/hSNF5 into other RT cells such as STA-WT1 and G401 also resulted in consistent downmodulation of Aurora A levels (Fig. 1C). These results indicated that INI1/hSNF5 downmodulates Aurora A gene expression in all RT cells tested.

To determine if INI1/hSNF5 can regulate Aurora A in non-RT cells, we knocked down endogenous INI1/hSNF5 in these cells. Expression of si-IN1I in non-RT cells such as Jurkats, CEMX-174, HeLa, Gliomas (SF268), 293T, and normal fibroblasts (CRL-2522 and BJ-TERT) resulted in 80% or greater reduction in the level of the protein (Fig. 1D, top). However, downmodulation of INI1/hSNF5 had neither no effect (as in Jurkats and HeLa) or a slight decrease (as in CEMX-174, Glioma and 293T) in Aurora A levels indicating that in non-RT cells, INI1/hSNF5 does not downregulate expression of Aurora A (Fig. 1D middle). Interestingly, downmodulation of INI1/hSNF5 increased Aurora A levels in 2 normal fibroblast cell lines (Fig. 1D, see CRL-2522 and BJ-TERT cells). These results indicated that INI1/hSNF5 selectively represses Aurora A gene in RT cells and in normal human fibroblasts.

Aurora A is a direct downstream target of INI1/hSNF5 in RT cells

To determine if INI1/hSNF5 affects Aurora A promoter function, we tested its effect on a reporter construct (pGL486) harboring luciferase gene under the control of ~1486 bp region upstream of Aurora A gene [Fig. 2A and ref. 23]. Expression of INI1/hSNF5 resulted in about 3-fold decrease of luciferase activity compared with that of empty vector control, indicating that INI1/hSNF5 represses transcription activity of a panel of Aurora A promoter deletion constructs (pGL415, pGL124, and pGL75). INI1/hSNF5 was able to repress transcription activity of each one of the constructs suggesting that minimal
region required for repression is within 75bp upstream region. This region harbors CDE (cell-cycle–dependent element) and CHR (cell-cycle gene homology region) elements, known to regulate cell-cycle–dependent expression of Aurora A gene. Mutation of CDE and CHR elements, however, did not alter INI1-mediated repression, indicating that the repression is independent on these sequences (Supplementary Fig. S1). Furthermore, HA-INI1 repressed transcription from pGL1486-Luc constructs in a panel of RT cells (MON, STA-WT1, and A204) and normal cells (CRL-2522 and BJ-TERT) but not in non-RT cells (HeLa and 293T; Fig. 2B). These results confirmed our hypothesis that endogenous INI1 is associated with Aurora A gene expression in normal cells and that INI1/hSNF5-mediated repression is specific to RT cells and is not observed in non-RT tumor cells.

To determine if INI1/hSNF5 is associated with Aurora A promoter, we carried out ChIP assay in MON RT cells transfected with HA-INI1 or control plasmid and immunoprecipitated with α-HA antibodies, control IgG, or no antibodies (Fig. 2C). The DNA isolated from immunoprecipitates was subjected to PCR amplification using primers specific to Aurora A promoter (see Fig. 2A). The results indicated that while α-HA antibodies were able to specifically immunoprecipitate Aurora A promoter region (Fig. 2C, top panel, lane 4), immune complex by IgG or no antibody controls did not show any amplification of Aurora A promoter region (Fig. 2C, lanes 2 and 3). As expected, α-HA antibodies failed to immunoprecipitate the Aurora A promoter region in cells transfected with empty vector (Fig. 2C, bottom panel). To determine if endogenous INI1 is associated with Aurora A promoter, we carried out ChIP assays in normal human fibroblast cells or control 293T using α-INI1 or si-INI1 antibodies. We found that α-INI1, but not control antibodies immunoprecipitated Aurora A promoter region specifically in normal fibroblast cells, but not in 293T cells (Fig. 2D). These results indicated that INI1/hSNF5 is associated with Aurora A promoter in a cell-type–specific manner, consistent with INI1-mediated cell-type–specific repression. These results indicated that Aurora A is a specific and direct downstream target of INI1/hSNF5 selectively in RT and normal cells.

**Derepression of Aurora A in human and mouse primary RTs harboring loss of INI1/hSNF5 gene**

Aurora A is a prognostic marker in many cancers. However, expression of Aurora A has never been investigated in RTs. Based on the above results, we surmised that loss of INI1/hSNF5 should lead to derepression of Aurora A gene in RTs. To test this hypothesis, 3 primary human RTs, 2 primary mouse RTs and mouse xenografted tumor derived from human G401 RT cell line, were subjected to: (i) quantitative real-time PCR...
using Aurora A, INI1, and GAPDH primers; and (ii) immunochemical analysis using α-Aurora A antibodies. The primary tumors were previously proven to have all characteristics of RTs including loss of INI1/hSNF5 (9). As shown in Figure 3A, all tumor samples showed several fold increase in Aurora A mRNA levels when compared with that of normal controls (Fig. 3A, left panel). In contrast to the elevated levels of Aurora A mRNA, the levels of INI1 mRNA were decreased in the tumor samples when compared with normal controls (Fig. 3A, right panel). Consistent with upregulation of Aurora A mRNA in the tumor samples, strong, and positive immunoreactivity of Aurora A was evident in primary human and mouse RT as well as xenografts (Fig. 3B). The staining was evident in all tumor cells including the interface cells and rhabdoid cells (Fig 3B, panel 17, pointed by arrows). However, normal brain tissues adjacent to these tumors were negative for α-Aurora A staining (Fig. 3B, panel 8). Staining the tumor samples with no antibody (negative control) showed no positivity for Aurora A (Fig 3B, panel 10). Furthermore, non-tumor stromal cells within RTs and cerebellar granular cells in the normal brain tissue adjacent to RTs remained immunonegative and served as internal controls (Fig. 3B panels 14 and 2 respectively, indicated by arrow heads).
analysis using α-INI1 antibodies showed the lack of INI1 expression in tumor cells but the presence of INI1 in stromal cells and cerebellar granular cells (Supplementary Fig. S2). These studies showed that Aurora A is derepressed/overexpressed, both at the mRNA and protein levels, in RT cells lacking INI1/hSNF5, consistent with our hypothesis that INI1/hSNF5 represses this gene in vivo.

**Knocking down of Aurora A inhibits RT cell growth and induces mitotic arrest in RT cells**

Based on the above results that Aurora A is a direct downstream target of INI1/hSNF5 in RT cells and that it is overexpressed/derepressed in primary RTs lacking INI1/hSNF5 expression, we hypothesized that Aurora A could be a useful therapeutic target for these tumors. To test this hypothesis, RNA interference analysis was used to knockdown Aurora A in RT cells. Immunoblot analysis indicated that treatment of RT cells with si-Aurora A resulted in downmodulation of Aurora A protein to more than 90% (Fig. 4A). Downmodulation of Aurora A by siRNA in 3 different RT cells, MON, G401, and STA-WT1, resulted in dramatic impairment of cell proliferation leading to significant reduction in cell number and altered and enlarged cell morphology compared with that of cells treated with control siRNA (si-control, Fig. 4B). These results show that downmodulation of Aurora A is deleterious to RT cell growth.

To further investigate the effects of downmodulation of Aurora A, MON cells treated with si-control or si-Aurora A were subjected to immunofluorescence analysis using α-tubulin antibody and DAPI (4',6-diamidino-2-phenylindole) for
cytoplasmic and nuclear staining. Confocal microscopic analysis indicated that cells treated with si-control harbor single normal kidney-shaped nuclei (Fig. 4C). On the contrary, cells transfected with si-Aurora A exhibited a dramatic alteration in cellular and nuclear morphology (Fig. 4C). The cells were enlarged and exhibited the presence of multiple and fragmented nuclei (Fig. 4C). Cells with multiple nuclei (>8) could be readily seen (Fig. 4C, panels 3 and 4). Furthermore, fragmented nuclei were apparent in cells transfected with si-Aurora A (Fig. 4C, panels 2). To quantify the severity of these effects of downmodulation of Aurora A, percentages of cells with fragmented or multiple nuclei were determined in si-control versus si-Aurora A treated cells (Fig. 4D). The results clearly indicated that si-Aurora A treatment resulted in dramatic increase of cells with fragmented as well as multiple nuclei compared with that of cells treated with si-control (Fig. 4D). These results indicated that RT cells are dependent on Aurora A expression for maintaining proper nuclear integrity and that downmodulation of this protein results in mitotic catastrophe.

**Downmodulation of Aurora A potently inhibits cell survival, and induces mitotic arrest and caspase 3/7-dependent apoptosis in RT cells**

To determine the effects of downmodulation of Aurora A on cell-cycle profile and cell survival in RT cells, we carried out fluorescence-activated cell sorting (FACS) analysis on cells treated with si-control or si-Aurora A. As shown in Figure 5A, while cells transfected with si-control exhibited a prominent peak with 2N DNA content, downmodulation of Aurora A
resulted in a peak with 4N DNA content. Furthermore, there was significant increase in cells with greater than 4N DNA content, consistent with increased fraction of multinucleated and aberrantly nucleated cells observed in immunofluorescence analysis (Fig. 5A and Fig. 4C). Previously, we had shown that treatment of siRNA to PLK1 in RT cells not only increased cells with greater than 4N DNA content but also induced cell death (14). To determine if knocking down PLK1 was cytotoxic to RT cells, we analyzed percentage of cells transfected with siRNA, as analyzed by FACS. C, percentage of cell viability of MON cells transfected with each siRNA, determined by MTS assay. D, induction of caspase 3 cleavage in cells transfected with si-Aurora or si-control. E, fold change in caspase 3/7 activity in cells transfected with indicated siRNA.

A comparative analysis of effect of downmodulation of Aurora A in RT, non-RT, and normal cells

Our results indicated that Aurora A and PLK1 are direct downstream targets of INI1/hSNF5 and that inhibiting expression of these genes has the potential to develop targeted therapy for RT [this report and ref. 14]. Downmodulation of PLK1 has a marginal or no effect on survival of normal diploid cell lines (38, 40). To determine the relative effects of downmodulating Aurora A versus PLK1 on cell viability of various RT, non-RT, and normal cell lines, we carried out a comparative study. Several RT and non-RT cells were subjected to siRNA treatment. Downmodulation of proteins was confirmed by immunoblot analysis (Fig. 6A). We carried out FACS analysis of panel of these cells to determine the relative degree of cell death induced by si-PLK1 or si-Aurora A, compared with
To determine the long-term effects of downmodulating Aurora A on RT cells, we generated lentiviral vectors expressing shRNA targeting Aurora A. The panel of RT and normal cells was transduced with lentiviral vectors carrying either control shRNA or Aurora A shRNA and survival rate of these cells were assessed over a period of several days. Immunoblot analysis indicated successful downmodulation of Aurora A in all cells transduced with lentiviral vectors expressing Aurora A shRNA (Fig. 6D). Analysis of these transduced cells using MTS assay indicated reduced survival upon downmodulation of Aurora A in all RT cells tested (Fig. 6E). Transduction by a second Aurora A shRNA, but not control shRNA, also resulted in decrease of survival of RT cells (Supplementary Fig. S3). However, knocking down Aurora A did not have any deleterious effects on normal cells even after long periods of time and with similar reduction of expression of Aurora A (Fig. 6D and E). These results indicated that in normal cells, although INI1 represses Aurora A, it does not lead to inhibition of survival. These results are consistent with the observation of lack of induction of apoptosis in normal cells upon Aurora A knockdown (Fig. 6C). These observations further confirmed that downmodulation of Aurora A is deleterious to RT cells but not to normal cells.

si-control. Our results indicated that knocking down either of these 2 proteins had minimal effect on inducing cell death in normal cells as assessed by analysis of subG1 population (Fig. 6B). Interestingly, knocking down Aurora A had most dramatic effects in RT cells, and induced 12% to 15% cell death compared with that in non-RT cells (Fig. 6B). We also tested the effect of knocking down Aurora A on induction of apoptosis in RT and normal cells. We found that cleaved caspase 3 products were specifically seen in RT cells, but not in normal cells, indicating that cell death induced upon Aurora knockdown is specific to RT cells (Fig. 6C).

Figure 6. Comparative analysis of effect of depletion of Aurora A in inducing apoptosis and defective cell proliferation in RT, non-RT, and normal cells. A, immunoblot analysis of RT (MON and STA-WT1), non-RTs (HeLa, 293T and Glioma), and normal (CRL-2522) cell lines transduced with control, PLK1, or Aurora A siRNA. B, fold change in the subG1 population of transfected cells depicted in A, as analyzed by FACS. C, induction of caspase 3 cleavage upon depletion of Aurora A in RT and non-RT cells. D and E, stable knockdown of Aurora A inhibits cell proliferation in RT cells, but not in normal cells. D, RT cells (MON, A204, G401, and STA-WT1) and normal cell (CRL-2522) were transduced with lentiviral vectors expressing shAurora A or control shRNA and subjected to immunoblot analysis. E, proliferation of 4 RTs and 1 normal fibroblast cells transduced with shRNAs and selected for puromycin, as measured by MTS assay for 7 days. Results are represented in optical density at 490 nm.
Discussion

We and other groups have shown that INI1/hSNF5 regulates multiple pathways and genes including cyclinD1/cdk/p16 pathway, mitotic checkpoint, interferon signaling and hedgehog signaling in RTs cells (11, 12, 14, 15, 41). Here, we have shown, for the first time, that INI1/hSNF5 represses Aurora A selectively in RT cells. Knocking down INI1/hSNF5 in fibroblasts upregulated Aurora A expression, implying that endogenous INI1/hSNF5 represses Aurora A in these cells. On the contrary, INI1/hSNF5 does not repress Aurora A in non-RT cells indicating that regulation of Aurora A by INI1 is cell-type specific.

The Aurora A promoter harbors a number of transcription factor binding sites within a 400 bp upstream region, including E2F, SP1, Ets, and CDE/CHR regions. The tandem repressor elements, CDE/CHR at −44 and −39, mediate repression of Aurora A (23). Our results indicated that mutating CDE/CHR elements did not significantly affect INI1/hSNF5-mediated repression, suggesting that this repression involves elements other than CDE/CHR. It is interesting to note that CDE/CHR elements mediate cell-cycle–dependent repression. Our results suggest that the repression mediated by INI1 is likely to be independent of stage of cell cycle. This is consistent with the observation that in primary RTs lacking INI1, Aurora A was upregulated independent of stage of cell cycle.

Chromatin immunoprecipitation assay indicated that INI1 is associated with Aurora A promoter in RT and normal cells where repression is observed, but not in non-RT (293T) cells where repression is not observed (Fig. 2D). INI1 and other components of SWI/SNF complex do not have a sequence-specific DNA binding activity, hence the complex needs to be recruited to the promoter elements via the protein–protein interaction of the components with gene-specific transcription factors. Based on the observation of cell-type–specific association of INI1 with Aurora A promoter, we suggest that perhaps non-RT cell lines are defective for some aspect of this promoter recruitment, resulting in no repression in these cells. Interestingly, INI1 represses Aurora A in normal cells in addition to RT cells, but INI1-mediated repression does not lead to mitotic catastrophe in normal cells. It is likely that the presence of other redundant mechanisms may compensate for the loss of Aurora A in normal cells.

Based on all the above observations, we propose that inactivation or loss of INI1/hSNF5 leads to overexpression of Aurora A (and other mitotic genes, such as PLK1), leading to improper regulation of mitotic spindle checkpoint, which could be one of the major causes of genesis of RT.

The Aurora A protein is a candidate target to inhibit the tumor growth in a broad range of cancers including pancreatic and leukemia cells in which it is amplified/overexpressed (19, 42). Based on our studies, it appears that transcriptional derepression of Aurora A because of loss of INI1/hSNF5 leads to overexpression of this protein in RTs. We suggest that targeting Aurora A could be effective in inhibiting RT growth. Downregulation of Aurora A by RNA interference induces cell-cycle arrest, aberrant chromosomal segregation and apoptosis, to a much higher efficiency than downregulation of PLK1 in RT cells. The normal diploid cells tolerate reduced expression of Aurora A, perhaps due to functional redundancies in mitotic genes. Our results also indicate that downregulation of Aurora A has a more dramatic effect in inducing apoptosis (about 4-fold higher) in RT cells than in HeLa and Glioma cells. Perhaps the survival of RT cells is more dependent on the activity of Aurora A, which is directly regulated by INI1/hSNF5. Taken together, Aurora A could be a promising therapeutic target in RTs. In support of our observations, pediatric preclinical testing program (PPTP) studies have indicated that small molecular weight Aurora A inhibitor (MLN 8327) has shown promising efficacy against RT xenografts in mice (personal communication with Drs. Peter Houghton and Malcolm Smith). We propose that a multi-targeted therapy that includes a regimen for targeting several genes, including Aurora A and cyclin D1, the 2 downstream effectors of INI1/hSNF5, will be valuable in dramatically inhibiting RT growth.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Aurora A Is a Repressed Effector Target of the Chromatin Remodeling Protein INI1/hSNF5 Required for Rhabdoid Tumor Cell Survival

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