Preclinical Development

The Aurora Kinase Inhibitor CCT137690 Downregulates MYCN and Sensitizes MYCN-Amplified Neuroblastoma In Vivo

Amir Faisal1, Lynsey Vaughan2, Vassilios Bavetsias1, Chongbo Sun1, Butrus Atrash1, Sian Avery1, Yann Jamin3, Simon P. Robinson3, Paul Workman1, Julian Blagg1, Florence I. Raynaud1, Suzanne A. Eccles1, Louis Chesler2, and Spiros Linardopoulos1,4

Abstract

Aurora kinases regulate key stages of mitosis including centrosome maturation, spindle assembly, chromosome segregation, and cytokinesis. Aurora A and B kinase overexpression has also been associated with various human cancers, and as such, they have been extensively studied as novel antimitotic drug targets. Here, we characterize the Aurora kinase inhibitor CCT137690, a highly selective, orally bioavailable imidazo[4,5-b]pyridine derivative that inhibits Aurora A and B kinases with low nanomolar IC50 values in both biochemical and cellular assays and exhibits antiproliferative activity against a wide range of human solid tumor cell lines. CCT137690 efficiently inhibits histone H3 and transforming acidic coiled-coil 3 phosphorylation (Aurora B and Aurora A substrates, respectively) in HCT116 and HeLa cells. Continuous exposure of tumor cells to the inhibitor causes multipolar spindle formation, chromosome misalignment, polyploidy, and apoptosis. This is accompanied by p53/p21/BAX induction, thymidine kinase 1 down-regulation, and PARP cleavage. Furthermore, CCT137690 treatment of MYCN-amplified neuroblastoma cell lines inhibits cell proliferation and decreases MYCN protein expression. Importantly, in a transgenic mouse model of neuroblastoma that overexpresses MYCN protein and is predisposed to spontaneous neuroblastoma formation, this compound significantly inhibits tumor growth. The potent preclinical activity of CCT137690 suggests that this inhibitor may benefit patients with MYCN-amplified neuroblastoma.

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Introduction

Neuroblastoma is the most common extracranial solid tumor of childhood, accounting for approximately 10% of pediatric tumors and affects more than 10,000 children worldwide each year. It originates from the sympathetic nervous system and is characterized by heterogeneous pathologic and clinical presentation. Stage 4 neuroblastoma represents approximately 50% of cases with metastatic dissemination at diagnosis and its prognosis is poor. Therefore, novel therapeutic strategies are urgently needed to improve the prognosis of patients with neuroblastoma. Amplification of the MYCN gene is associated with an aggressive form of neuroblastoma that results in a particularly poor clinical outcome (1). Down-regulation of MYCN protein by targeting with short interfering RNA (siRNA), or alternatively, destabilizing the protein with an inhibitor of the upstream phosphoinositide 3-kinase signaling pathway, has been shown as an effective preclinical therapy for neuroblastoma (2, 3). Furthermore, an association between MYCN and Aurora kinases has also been reported; a synthetic lethal screen identified AURKA as a gene required for the growth of MYCN-amplified neuroblastoma cell lines. In addition, Aurora A was shown to stabilize MYCN protein through its physical interaction with MYCN and the E3 ubiquitin ligase FBXW7 (4). A recent study using MLN8237, a small-molecule inhibitor of Aurora A kinase, has also shown efficacy against neuroblastoma in preclinical models (5) Therefore, inhibition of the Aurora kinases may be an effective strategy to treat MYCN-amplified neuroblastoma.

The Aurora kinases are a family of highly conserved serine/threonine kinases that are important for faithful transition through mitosis (6–8). Their activity and protein expression are cell-cycle regulated, peaking during mitosis to orchestrate important mitotic processes
including centrosome maturation, chromosome alignment, chromosome segregation, and cytokinesis (6, 8, 9). In humans, the Aurora kinase family consists of 3 members, Aurora A, Aurora B, and Aurora C, that each share a conserved C-terminal catalytic domain but differ in their subcellular localization, substrate specificity, and function during mitosis (9, 10). At the onset of mitosis, Aurora A binds to TPX2 and is activated by autophosphorylation at threonine 288 (T288). Once activated, Aurora A phosphorylates multiple substrates that regulate centrosome maturation and mitotic spindle assembly (11, 12). Aurora B is part of the chromosomal passenger complex along with inner centromere protein, survivin, and borealin. Aurora B is activated through binding and phosphorylation of inner centromere protein in a positive feedback mechanism (13). Because the phosphorylation of histone H3 at serine 10 (S10) and serine 28 (S28) by Aurora B is required for chromosome condensation (14), inhibition of S10 phosphorylation has been widely used as a biomarker for Aurora B inhibition in vitro and in vivo (15). Aurora C is also a chromosomal passenger protein, but unlike Aurora A and B, its expression is restricted to testes (16).

The amplification and overexpression of Aurora A and Aurora B have been reported in many human tumors, including breast, colon, pancreatic, and ovarian cancers and neuroblastoma, with high Aurora A expression levels being associated with poor prognosis and genomic instability (17, 18). These properties have made the Aurora kinases attractive antimitotic drug targets and as such a number of Aurora kinase inhibitors are currently being evaluated preclinically and in clinical trials (16). We have recently identified CCT137690 as a potent and highly selective Aurora kinase inhibitor, described its mode of binding to Aurora A kinase, and shown its in vitro efficacy in xenografts in mice using human cancer cell lines (19). In addition to inhibiting the Aurora kinases, we have recently identified CCT137690 as a potent inhibitor of oncogenic FLT3 and showed its in vitro and in vivo activity in FLT3-ITD–positive acute myeloid leukemia (20).

Aurora kinase inhibitors, therefore, represent new agents for the treatment of a variety of cancers including neuroblastoma, particularly when associated with MYCN gene amplification. Here, we report an extensive cellular characterization and the potent preclinical activity of CCT137690 in MYCN-amplified neuroblastoma in vitro and in vivo.

MATERIALS AND METHODS

Plasmids and reagents

Myc-tagged Aurora A cloned in pCR2.1 vector has been previously described (21). pCMV expression vector for human transforming acidic coiled-coil 3 (TACC3) was a kind gift from Dr. Fenni Gergely (Cambridge Research Institute, Cambridge, United Kingdom). Synthesis of CCT137690, an imidazo[4,5-b]pyridine derivative, has been described elsewhere (19). Rabbit polyclonal antibodies against TACC3 phosphorylated at serine 558 (S558) were raised against phospho-peptide TADL-DIPJITEINK (Eurogentech). Other antibodies used were phospho-Aurora A (T288), total Aurora A, BAX, cleaved PARP, phospho-GSK3β (Cell Signaling), phospho-histone H3 (Millipore), total histone H3 (Abcam), p21, p53, myc, MYCN (Santa Cruz), tubulin (Sigma), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Chemicon International).

Cell culture, transfections, and immunoblotting

All cell lines were purchased from the American Type Culture Collection except the SHEP, which was obtained from the University of California at San Francisco Cell Culture Facility, and KELLY, which was purchased from the Health Protection Agency Culture Collections. All cell lines were grown in their recommended culture medium, supplemented with 10% FBS at 37°C in 5% CO2, and passaged for less than 6 months before replacement from early passage frozen stocks. Cells were transfected at approximately 80% confluence with the plasmids indicated, using Lipofectamine LTX (Invitrogen) according to the manufacturer’s instructions. For immunoblotting, cells were collected either in NP-40 lysis buffer (120 mmol/L NaCl, 50 mmol/L Tris HCl, pH 7.5, 1% NP-40 supplemented with phosphatase and protease inhibitors) or 2 × LDS sample buffer (Invitrogen). Equal amounts of proteins were resolved by 4% to 12% Bis-Tris NuPAGE gels (Invitrogen), transferred to nitrocellulose (Whatman) membranes, and immunoblotted with specific antibodies. Cells were regularly screened for Mycoplasma, using a PCR-based assay (VenorGem; Minerva Biolabs).

Cell proliferation assays

Cell proliferation assays were carried out by colorimetric MTT method (Sigma) as described elsewhere (22). Briefly, cells were plated in 96-well plates at 3,000 cells per well and were treated with a range of 0 to 25 mol/L CCT137690 for 72 hours. The absorbance was measured at 570 nm with the Wallac VICTOR2 1420 Multilabel Counter (PerkinElmer).

Cell-cycle and immunoﬂuorescence analyses

Cell-cycle analysis was conducted as described previously (22). Briefly, cells were treated with different concentrations of CCT137690 for 24, 48, and 72 hours, harvested with 5 mmol/L EDTA-PBS, and fixed in 85% ice-cold ethanol. Fixed cells were then stained with propidium iodide (Sigma)/RNase solution for 30 minutes at 37°C and analyzed on Beckman Coulter Cytomics FC500. For immunoﬂuorescence, cells were grown on coverslips in 24-well tissue culture plates, treated with CCT137690 for 4, 24, or 48 hours, and fixed with 4% formaldehyde for 15 minutes at room temperature or with ice-cold methanol for 15 minutes at −20°C (for phospho-Aurora-A-T288). Cells were then permeabilized and blocked with 2% bovine serum albumin (BSA) in PBS containing 0.1% Triton X-100 before incubation with...
primary antibodies for 1 hour at room temperature (1:100 dilutions in 2% BSA). Cells were washed 3 times with PBS and incubated with appropriate Alexa 488 or 568 antibodies for 45 minutes (1:1,000 dilution in 2% BSA) and washed 3 times with PBS. To visualize nuclei, TO-PRO-3 iodide (Invitrogen; 1:10,000) was added during the last wash. Coverslips were mounted on glass slides with Mowiol (Polysciences), and fluorescence was visualized with a Leica SP1 confocal microscope.

**In vivo efficacy studies**

Animals were randomized into 2 groups: group 1, treatment with 100 mg/kg CCT137690, \( n = 4 \); group 2, vehicle control, \( n = 4 \). Treatment was administered via oral gavage twice daily. Tumor volumes were measured at day 0, 3 (48 hours after treatment started), 7, and 10 using \( ^{1}H \) MRI. \( ^{1}H \) MRI was carried out on a 7T Bruker horizontal bore microimaging system equipped with a 3-cm birdcage coil. Anesthesia was induced by an intraperitoneal injection of a combination of fentanyl citrate (0.315 mg/mL) plus fluanisone (10 mg/mL; Hypnorm; Janssen Pharmaceutical), midazolam (5 mg/mL; Roche), and water (1:1:2). Anatomic T2-weighted images were acquired from 20 contiguous 1-mm thick coronal slices through the mouse abdomen with a 240-\( ^{1}H \) in-plane resolution (RARE sequence; NEX = 4; TR = 36 milliseconds; TE = 4,500 milliseconds; turbo factor = 8; 1 mm thick contiguous; matrix = 128 × 128). Tumor volume was measured by segmentation from regions of interest drawn on every slice-containing tumor, using in-house software Imageview (written under IDL programming platform; ITT). Significance analysis was done by the Student \( t \) test. CCT137690 was quantified in extracted plasma and tumor samples by high-performance liquid chromatography with tandem mass spectrometry, using reverse-phase gradient elution chromatography and multiple reaction monitoring. All animal studies were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and National Guidelines (23).

**Results**

**CCT137690, a selective Aurora kinase inhibitor, potently inhibits proliferation of human tumor cell lines**

Our lead optimization studies to develop a potent, highly selective, and orally bioavailable Aurora kinase inhibitor resulted in the discovery of CCT137690 (Fig. 1), an imidazo[4,5-b]pyridine that inhibits Aurora A, B, and C kinases with \( IC_{50} \) values of 0.015, 0.025, and 0.019 \( \mu \)mol/L, respectively (19). CCT137690 is highly selective and inhibited the activity of 3 additional kinases, namely, FLT3 (\( IC_{50} \) 0.0025 \( \mu \)mol/L), FGFR1, and VEGFR above 80% at 1 \( \mu \)mol/L in a panel of 94 kinases (19). Here, we evaluated the antiproliferative activity of CCT137690 against a wide range of human tumor cell lines, using an MTT assay. CCT137690 effectively inhibited the growth of human tumor cell lines of different origins with \( GI_{50} \) (growth inhibition by 50%) values ranging from 0.005 to 0.47 \( \mu \)mol/L (Table 1). Within this panel of cell lines, we did not find an obvious relationship between sensitivity to growth inhibition and the protein levels of the Aurora kinases. Differences in sensitivity might rather be associated with different genetic backgrounds of the cell lines (e.g., lack of proteins associated with Aurora kinases; ref. 22).

**CCT137690 inhibits both Aurora A and B in cells**

We next investigated the ability of CCT137690 to inhibit Aurora A autophosphorylation at T288 and histone H3 phosphorylation at S10 (an Aurora B substrate) in HeLa cells. Both the autophosphorylation of ectopically expressed Aurora A and the phosphorylation of histone H3 (pHH3) were efficiently inhibited by CCT137690 in a dose-dependent manner (Fig. 2A), with complete inhibition being seen for both P-T288 and pHH3 at 0.5 \( \mu \)mol/L.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>CCT137690 ( GI_{50} ) ( \mu )mol/L</th>
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<tbody>
<tr>
<td>SW48</td>
<td>0.005</td>
</tr>
<tr>
<td>T84</td>
<td>0.14</td>
</tr>
<tr>
<td>SW620</td>
<td>0.15</td>
</tr>
<tr>
<td>Ls174T</td>
<td>0.16</td>
</tr>
<tr>
<td>SW403</td>
<td>0.18</td>
</tr>
<tr>
<td>SW948</td>
<td>0.19</td>
</tr>
<tr>
<td>HCT116</td>
<td>0.22</td>
</tr>
<tr>
<td>DLD1</td>
<td>0.26</td>
</tr>
<tr>
<td>Colo320</td>
<td>0.36</td>
</tr>
<tr>
<td>PC/JW2</td>
<td>0.45</td>
</tr>
<tr>
<td>LOVO</td>
<td>0.47</td>
</tr>
<tr>
<td>A2780</td>
<td>0.35</td>
</tr>
<tr>
<td>HeLa</td>
<td>0.14</td>
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Figure 1. CCT137690 is a potent pan-Aurora kinase inhibitor with antiproliferative activity against a number of human tumor cell lines. Structure of CCT137690.
CCT137690. The blots were quantified by densitometry (Image J software; NIH), and the IC50 values were calculated to be 0.025 and 0.093 μmol/L for P-T288 and pHH3, respectively. The phosphorylation of TACC3 by Aurora A at S558 is required for its localization at centrosomes and proximal microtubules and has been used as a biomarker for Aurora A inhibition in cells (24, 25). We have generated and characterized an antibody against TACC3 phosphorylated at S558 (Supplementary Fig. S1). To test whether CCT137690 inhibits TACC3 phosphorylation, cells overexpressing TACC3 and Aurora A were treated with increasing concentrations of CCT137690 for 2 hours following overnight treatment with nocodazole followed by treatment with different concentrations of CCT137690 for 2 hours. TACC3 phosphorylation at S558 and histone H3 phosphorylation at S10 were analyzed by immunoblotting. Total TACC3 and total histone H3 (Total-H3) were used as loading controls. C, immunofluorescence of HeLa cells treated with 50 ng/mL nocodazole overnight, followed by treatment with CCT137690 for 24 hours. Cells were fixed and stained with pH3 S10 antibody. D, immunofluorescence of HeLa cells were treated with CCT137690 for 4 hours, fixed, and stained with P-T288 antibody. 4’,6-Diamidino-2-phenylindole staining (blue) indicates the DNA content (bar, 50 μm). DMSO, dimethyl sulfoxide.

CCT137690 induces polyploidy, mitotic aberrations, and apoptosis in tumor cells

Aurora B plays an important role in cytokinesis, and its inhibition results in the accumulation of cells with 4N or more DNA content (22, 26). We tested whether CCT137690 induces a similar phenotype in HCT116 cells. As shown in Fig. 3A, treatment of HCT116 cells with 0.5 or 1 μmol/L CCT137690 for 24 hours induced accumulation of cells with 4N and 8N DNA content, indicating cytokinesis failure and induction of polyploidy. Moreover, following 48 and 72 hours of treatment with CCT137690, cells with 16N DNA content can also be observed (Fig. 3A). A similar induction of 4N or more cell population by CCT137690 was observed in HeLa cells (Supplementary Fig. S2). To test the effects of CCT137690 on mitosis, HeLa cells were treated with 0.5 and 1 μmol/L of CCT137690 for 24 and 48 hours and
then analyzed by immunofluorescence. As shown in Fig. 3B, treatment with CCT137690 for 24 hours induced multipolar spindle formation and misaligned chromosomes. In addition, when using 0.5 μmol/L of the inhibitor, monopolar spindles were observed in the cells, consistent with Aurora A inhibition. Extension of the treatment to 48 hours resulted in multiple aberrant spindles, indicative of polyploid cells. Continuous exposure of HCT116 cells to CCT137690 for up to 72 hours resulted in increased levels of p53 and the proapoptotic protein BAX and PARP cleavage (Fig. 3C; Supplementary Fig. S3). Treatment of HCT116 cells with 0.5 μmol/L CCT137690 for 24 hours upregulated p53 and p21 and downregulated thymidine kinase 1 (TK1; Supplementary Fig. S4), confirming previously reported modulation of these biomarkers by Aurora inhibition that may be used in a noninvasive positron emission tomographic imaging to monitor the activity of Aurora inhibitors in vivo (22).

CCT137690 inhibits growth of neuroblastoma cell lines with MYCN amplification

Aurora A has been shown to stabilize MYCN by altering FBXW7-induced ubiquitin-mediated proteolytic degradation (4). Therefore, we first tested whether CCT137690 specifically sensitizes the growth of MYCN-amplified human neuroblastoma cell lines. A panel of neuroblastoma cell lines expressing different levels of MYCN was treated with CCT137690. Neuroblastoma cell lines with increased levels of MYCN (KELLY, SH-SY5Y, IMR32, and SHEP with stable expression of exogenous wild-type MYCN) showed higher sensitivity to CCT137690 than those with low- or no-
detectable MYCN expression (SK-N-SH and SHEP; Table 2). Treatment of KELLY, an MYCN-amplified neuroblastoma cell line, with CCT137690 resulted in a reduction of both p-T288 Aurora A and pHH3 at nanomolar doses, indicative of the inhibition of Aurora A and Aurora B, respectively (Fig. 4A). Similar inhibition of pHH3 by CCT137690 was observed in 4 cell lines with different levels of MYCN (Supplementary Fig. S5), indicating that the difference in sensitivity of these cell lines to CCT137690 is not due to differential inhibition of Aurora kinases but possibly due to differences in MYCN levels. To assess the effect of CCT137690 on MYCN protein levels, KELLY cells were treated with different concentrations of CCT137690 for 6 or 24 hours, stimulated with 50 ng/mL insulin-like growth factor 1 before harvesting and analyzed by immunoblotting. As shown in Fig. 4B, treatment with 3- and 6-fold the GI50 concentration of CCT137690 at both 6 (left) and 24 hours (right) resulted in a decrease in MYCN levels. Reduction of MYCN levels also correlated very closely with decreasing levels of GSK3β phosphorylation, as indicated by serine 9 (S9) phosphorylation of GSK3β (Fig. 4C). The phosphorylation of GSK3β at S9 by Aurora A kinase is inactivating (27, 28). Active GSK3β phospho- MYCN at Thr58, which is required for its recognition by the E3 ubiquitin ligase FBXW7, resulting in MYCN ubiquitination and proteolytic degradation (29). To test whether CCT137690-mediated downregulation of MYCN and inhibition of GSK3β phosphorylation at S9 was due to Aurora A inhibition, KELLY cells overexpressing Aurora A were treated with CCT137690. Overexpression of Aurora A in KELLY cells increased both MYCN protein levels and GSK3β phosphorylation at S9 compared with the vector control (Fig. 4C). Aurora A inhibition by CCT137690 reduced both MYCN expression and S9 GSK3β phosphorylation (Fig. 4C). These data indicate that the downregulation of MYCN is due to Aurora A inhibition by CCT137690.

**Discussion**

The Aurora kinases are serine/threonine kinases that play essential roles during mitosis (6, 10). In addition, the Aurora kinases are amplified and overexpressed in a wide variety of cancers and their upregulation often correlates with poor prognosis. Therefore, they have been extensively studied as potential therapeutic targets in solid tumors and hematopoietic cancers (6, 17, 18, 31). In this study, we further characterized the novel Aurora kinase inhibitor CCT137690 (19). The compound showed considerable selectivity to Aurora kinases among a panel of kinases tested and inhibited the growth of a wide range of human tumor cell lines. This sensitivity might be associated with the inhibition of all Aurora kinases, in particular Aurora A and Aurora B, in addition to a different genetic background among the cell lines. Our results, consistent with previous studies, showed that inhibition of Aurora kinases by CCT137690 resulted in stabilization of p53, p21, and the proapoptotic protein BAX in tumor cells. This led to PARP cleavage and apoptosis. Our data also confirmed that CCT137690 downregulates TK1. Noninvasive 3′-deoxy-

<table>
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<th>Cell line</th>
<th>Mean CCT137690 GI50 (μmol/L; n = 3) at 72 h</th>
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<tr>
<td>KELLY (MYCN Ampl+)</td>
<td>0.33</td>
</tr>
<tr>
<td>IMR32 (MYCN Ampl+)</td>
<td>0.38</td>
</tr>
<tr>
<td>SHEP WT (SHEP with stable expression of WT MYCN)</td>
<td>0.39</td>
</tr>
<tr>
<td>SH-SYSY (high MYCN protein expression)</td>
<td>0.92</td>
</tr>
<tr>
<td>SK-N-SH (low MYCN protein expression)</td>
<td>4.93</td>
</tr>
<tr>
<td>SHEP (no detectable levels of MYCN protein)</td>
<td>9.21</td>
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</table>

NOTE: Both SH-SYSY and SHEP cell lines are derived from parental SK-N-SH. Abbreviation: WT, wild type.
3-\textsuperscript{[18F]}fluorothymidine positron emission tomographic imaging, as previously reported, may therefore be used to measure the activity of CCT137690 \textit{in vivo} (22).

Aurora A and Aurora B have been shown to phosphorylate the centrosomal protein TACC3 at S558 and histone H3 at S10, respectively (24, 32). Consistent with this, we showed that CCT137690 potently inhibits the phosphorylation of both substrates in tumor cells. CCT137690 treatment of human colon cancer HCT116 cells caused cytokinesis failure that leads to abnormal mitotic spindles and cells with 4N or more DNA content. The observed abnormal mitotic spindles and multinucleation of tumor cells treated with CCT137690 resemble the phenotype induced in cells by inhibiting Aurora A and Aurora B using short interfering RNA or other chemical inhibitors (33, 34).

Neuroblastoma is one of the deadliest solid tumors in children (35). Up to 60% of patients present with widely metastatic disease (more often bone and bone marrow metastases), with a poor long-term survival rate of less than 10% (35) despite intensive treatments including surgery, chemotherapy, and irradiation. Thus, a search for novel, more potent, and less toxic treatments of neuroblastoma is currently underway. The particularly aggressive subtype of neuroblastoma characterized by MYCN amplification represents approximately 20% of total cases. Aurora A has been previously suggested to cause MYCN stabilization in human neuroblastoma by inhibiting FBXW7-induced ubiquitination and degradation (4). It was suggested that the stabilization of MYCN was due to the direct interaction with Aurora A that inhibited its proteosomal degradation in a kinase-independent manner. In more detail, Aurora A was shown to promote the synthesis of non-K48-linked ubiquitin chains, thereby inhibiting FBXW7-induced ubiquitination and degradation of MYCN. There is however...
the phosphorylation/inactivation of the GSK3 
CCT137690 treatment reduced the levels of MYCN and 
ma cell lines are more sensitive to CCT137690. 
MYCN protein and GSK3 

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Growing evidence, as shown in this study and in other recent studies (5, 36), that neuroblastoma cells are highly sensitive to Aurora kinase inhibitors both in vitro and in vivo. Whether Aurora kinase inhibitors organize Aurora A protein in a structural conformation that destabilizes its interaction with MYCN still remains to be answered. It would be interesting to study whether similar mechanisms control the expression levels of other members of the MYC family, such as c-Myc, which are also targets of degradation by the ubiquitin ligase FBXW7. In a previous study, we have shown that inhibition of Aurora kinases by the small-molecule inhibitor CCT137202 reduced the transcriptional levels of c-Myc, as shown by expression array analysis (22). Therefore, there are at least 2 mechanisms through which the Aurora kinases control the expression of the MYC genes, one at the transcriptional and another at the posttranslational level. These observations strongly suggest that Aurora inhibitors would significantly enhance the treatment of Myc-dependent cancers. Further studies using selective inhibitors toward Aurora A will be required to determine whether the observed destabilization of MYCN is primarily due to its ubiquitination and proteolytic degradation, stimulated by Aurora A inhibition, or whether this occurs by alternative mechanisms.

Here in this study, using the novel Aurora kinase inhibitor CCT137690, we identified the Aurora kinases as critical protein targets in neuroblastoma cell lines and tumors. We showed that MYCN-amplified neuroblastoma cell lines are more sensitive to CCT137690. CCT137690 treatment reduced the levels of MYCN and the phosphorylation/inactivation of the GSK3β kinase. Furthermore, CCT137690-mediated downregulation of MYCN protein and GSK3β phosphorylation is specific-

ly due to Aurora A inhibition. Importantly, CCT137690 dramatically reduces neuroblastoma tumor mass in MYCN transgenic mice when used as a single agent. In summary, CCT137690 is a highly selective, orally available Aurora kinase inhibitor with potent in vitro and in vivo efficacy in MYCN-amplified neuroblastomas.

Disclosure of Potential Conflicts of Interest

All authors are employees of The Institute of Cancer Research that has a commercial interest in drug development programs (see www.icr.ac.uk). Note that all authors who are, or have been, employed by The Institute of Cancer Research are subject to a “Rewards to Inventors Scheme,” which may reward contributors to a program that is subsequently licensed.

Acknowledgments

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References


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Molecular Cancer Therapeutics

The Aurora Kinase Inhibitor CCT137690 Downregulates MYCN and Sensitizes MYCN-Amplified Neuroblastoma In Vivo


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