A Cancer-associated Aurora A Mutant Is Mislocalized and Misregulated Due to Loss of Interaction with TPX2*

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Mutations in protein kinases can drive cancer through alterations of the kinase activity or by uncoupling kinase activity from regulation. Changes to protein expression in Aurora A, a mitotic Ser/Thr kinase, are associated with the development of several human cancers, but the effects of somatic cancer-associated mutations have not been determined. In this study we show that Aurora A kinase activity is altered in different ways in three somatic cancer-associated mutations located within the catalytic domain; Aurora A(V174M) shows constitutively increased kinase activity, Aurora A(S155R) activity is decreased primarily due to misregulation, and Aurora A(S361*) activity is ablated due to loss of structural integrity. These alterations suggest vastly different mechanisms for the role of these three mutations in human cancer. We have further characterized the Aurora A(S155R) mutant protein, found that its reduced cellular activity and mislocalization are due to loss of interaction with TPX2, and deciphered the structural basis of the disruption at 2.5 Å resolution. Previous studies have shown that disruption of the Aurora A/TPX2 interaction results in defective spindles that generate chromosomal abnormalities. In a panel of 40 samples from microsatellite instability-positive colon cancer patients, we found one example in which the tumor contained only Aurora A(S155R), whereas the normal tissue contained only wild-type Aurora A. We propose that the S155R mutation is an example of a somatic mutation associated with this tumor type, albeit at modest frequency, that could promote aneuploidy through the loss of regulated interactions between Aurora A and its protein partners.

Aurora A is a centrosome and mitotic spindle-associated, cell cycle-regulated serine/threonine kinase and is a key regulator of mitosis (1–5). The protein levels and the activity of Aurora A peak at G2 and during mitosis, whereas its expression is low in resting cells (1, 6). The Aurora A gene is located on human chromosome 20q13, a region that has been found to be amplified in a variety of human tumors (7). Overexpression of Aurora A induces abnormal spindle formation, leading to prolonged mitosis and polyploidy (8). Aurora A was first described in human cancer cell lines but has subsequently been found to be overexpressed in a broad range of human tumors, including primary colorectal carcinoma, gliomas, breast, ovarian, and pancreatic cancers (1, 9–12).

The association of Aurora A with cancer has prompted many studies that have revealed details of the function and regulation of the kinase. Bona fide substrates that are regulated by Aurora A phosphorylation include TACC3, Plk1, Eg5, and p53 (13–22). The catalytic activity of Aurora A is activated by phosphorylation on Thr-288 in its activation loop and by interaction with partner proteins such as TPX2, Ajuba, and HEF1 (13, 23–27). Aurora A is deactivated by dephosphorylation of Thr-288 by protein phosphatase 1 (PP1), 4 which is prevented by TPX2 (24, 25, 27). Aurora A and TPX2 are both degraded by the proteasome after APC/C-mediated ubiquitination (28, 29). TPX2 binds to and localizes Aurora A to spindle microtubules, including microtubules on the periphery of spindle poles (26). The centrosomal localization of a second population of Aurora A is independent of TPX2, and at least in Drosophila depends on the centrosomin protein (30).

Mutations in Aurora A have been identified that are associated with cancer. For example, the Aurora A(F31I) polymorphism shows preferential amplification associated with increased aneuploidy in colon cancers and is a low penetrance cancer susceptibility allele affecting multiple cancer types (31, 32). The Cancer Genome Project identified three somatic mutations in Aurora A within the catalytic domain; they are two single site missense mutations (Aurora A(V174M) and Aurora A(S155R)) and also one non-sense mutation (Aurora A(S361*)), which produces a C-terminal-truncated protein (33). The mutations are not located in well known driver hot-spot locations within the kinase structure, and therefore, it is essential to determine their molecular effects.

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†The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3.
‡The atomic coordinates and structure factors (code 2WQE) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).
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4 The abbreviations used are: PP1, protein phosphatase 1; MBP, myelin basic protein; GFP, green fluorescent protein; Bis-Tris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol; PBS, phosphate-buffered saline.
To gain further insights into the nature of the somatic Aurora A mutations, we examined the biochemical and functional characteristics of Aurora A(V174M), Aurora A(S155R), and Aurora A(S361*) mutants. Here we demonstrate that the Aurora A(V174M) mutant showed increased kinase activity relative to the wild type, whereas the Aurora A(S361*) mutation abolished activity. The Aurora A(S155R) mutant kinase activity is reduced compared with the wild type. In addition, Aurora A(S155R) does not bind TPX2 and is localized only at the centrosomes in mitotic cells. We present crystallographic analysis of the Aurora A(S155R) kinase domain that explains the abolished binding of Aurora A to TPX2 due to local rearrangements of the protein structure that sterically hinder binding.

**EXPERIMENTAL PROCEDURES**

**Cloning, Expression, Purification, and Lentiviruses**—Aurora A(S155R) and Aurora A(V174M) mutations were generated in wild-type Aurora A amino acids 122–403 (pETM11) using QuikChange site-directed mutagenesis (Stratagene) according to the manufacturer’s protocols, and DNA sequencing (MWG) was used to confirm success. Aurora A and TPX2 were expressed and purified as in Bayliss et al. (24). Aurora A(S155R) and Aurora A(V174M) were confirmed to be phosphorylated on Thr-288 by Western blot using a phosphospecific antibody (Cell Signaling Technologies). PP1α was expressed and purified as in Zhuo et al. (34). A human Myc-tagged wild-type, Aurora A(V174M), Aurora A(S155R), and Aurora A(S361*) cDNA was PCR-amplified using the following primers containing a Pmel restriction site: Aurora A forward, 5'-GTGTTAACATGGGAG-CAGAAGCTG-3', and Aurora A reverse, 5'-GTTTAAAC-GCTAAGACTGTTTGCT-3'. The amplification product was inserted into the pCR2.1 cloning vector (Invitrogen). The cDNA was released by Pmel digestion before insertion into Pmel-linearized pWPI lentiviral expression plasmid (Trono lab). Wild-type Aurora A and mutant lentiviruses were produced after cloning into the pWPI bicistronic expression vector containing the GFR marker using the Tronolab system of co-transfecting pWPI-Aurora A wild-type/mutants, psPAX2 packaging, and pMD2.G envelope plasmids into 293T packaging cells. Supernatant containing the virus was harvested 48, 72, and 96 h post-transfection and filtered via 0.45-µm filters before freezing at −80 °C. Titration was performed by flow cytometry of infected cells for GFP expression. Cells were infected by adding viral particles to the growth medium and incubating for at least 48 h.

**In Vitro Kinase Assay**—1.25 µM wild-type Aurora A, Aurora A(S155R), Aurora A(V174M), and Aurora A(D274N) in the presence or absence of wild-type TPX2 1–43 was used to measure kinase activity in vitro at an Aurora A:TPX2 ratio of 1:0.5, 1:1, 1:2, and 1:5. 11.25 µl of master mix containing 1.25 µl of 10× kinase assay buffer (500 mM Tris, pH 7.5, 100 mM NaCl, 25 mM MgCl₂, and 10 mM dithiothreitol), 1.25 µl of 100 µM ATP (Sigma), 1.25 µl of 5 mg/ml myelin basic protein (MBP; Sigma) as a substrate plus 0.1 µl of 10 µCi/µl [γ-32P]ATP (PerkinElmer Life Sciences) was added to 1.25 µl of enzyme with or without TPX2 to a final volume of 12.5 µl. Samples were incubated in a thermomixer at 25 °C for 10 min at 800 rpm. The reaction was stopped by the addition of 5 µl of 4× sample buffer plus 10% β-mercaptoethanol and heating at 80 °C for 3 min. Samples were then separated by SDS-PAGE, dried for 1 h at 80 °C, and exposed to x-ray film. Band intensity was quantified using ImageJ software.

In **In Vitro Pulldown Assays**—Saturating levels of wild-type glutathione S-transferase-TPX2-(1–43) or Δ1D/F16D-TPX2-(1–43) (350 µg) were bound to 25 µl of glutathione resin (Invitrogen) in 1 ml of binding buffer (PBS, 150 mM NaCl, 5 mM MgCl₂, 10 mM imidazole, 10% glycerol, 0.5% Tween 20) and incubated for 1 h at 4 °C with rotation. Beads were washed 3 times, and 35 µg of wild-type Aurora A, Aurora A(S155R), or Aurora A(V174M) was added. After binding for 1 h at 4 °C with rotation, the resin was added to a SPIN-X 0.22-µm cellulose acetate centrifuge filter (Coster) and centrifuged at 20,000 × g for 2 min. The flow-through was collected, and the resin was washed 3 times. 10 µl of beads or flow were analyzed by SDS-PAGE and Western blot using a phosphospecific antibody Thr(P)-288 Aurora A (Cell Signaling Technologies).

**Dephosphorylation Protection Assays**—200 µM wild-type Aurora A, Aurora A(D274N), Aurora A(S155R), and Aurora A(V174M) amino acids 122–403-His₆ was incubated in phosphatase buffer (50 mM Tris, pH 7.5, 0.1 mM EDTA, 2 mM MnCl₂, 5 mM dithiothreitol, 0.025% Tween 20) in the presence or absence of 0.2 µM PP1α and/or 38 µM TPX2 at 30 °C for 60 min. The reaction was stopped by the addition of boiling SDS loading buffer. Proteins were then separated by SDS-PAGE and analyzed by Western blot using α-Aurora A-Thr(288)-288 or α-PP1α (Cell Signaling Technologies).

**Tissue Culture**—293T and HeLa cells were maintained in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum, 2.4 mM l-glutamine, penicillin, and streptomycin at 37 °C, 5% CO₂.

**Immunoprecipitation Kinase Assay**—293T cells (6 × 10⁶/10 cm dish) were transfected with Myc-epitope-tagged wild-type Aurora A, Aurora A(S155R), Aurora A(V174M), and Aurora A(S361*) or empty vector (Myc-tagged pCMV). Transfection was carried out simultaneously with cell seeding using Lipofectamine 2000 (Invitrogen) and incubated for ~30 h before treatment with 50 ng/ml nocodazole (Sigma) for 16 h before preparation of protein extracts. Cells were harvested and suspended in lysis buffer (0.5% Igepal CA-630, 250 mM NaCl, and 50 mM HEPES, pH 7.4) containing protease and phosphatase inhibitors (complete protease inhibitor, Roche Applied Science, 1 mM dithiothreitol, 1 mM EDTA, 1 mM NaF, 10 mM β-glycerophosphate, 0.1 mM sodium orthovanadate) and incubated for 1 h at 4 °C. Samples were centrifuged at 20,000 × g for 10 min at 4 °C, and supernatant was collected. Protein concentration was determined by the BCA assay (Pierce). Cell lysate was precleared with protein A/G-agarose beads (Santa Cruz) for 1 h with rotation. Aurora A immunoprecipitation was performed at 4 °C using 40 µl of c-Myc-agarose conjugate slurry (clone 9E10, Santa Cruz), 1 mg of total protein per sample, and 1% bovine serum albumin (Sigma) for 2 h with rotation. Beads were washed extensively with lysis buffer then with kinase assay buffer (50 mM Tris, pH 7.5, 10 mM NaCl, 2.5 mM MgCl₂, and 1 mM dithiothreitol). 20 µl of kinase reaction mix containing 5 µl of 5 mg/ml MBP (Sigma) as a substrate, 2.5 µl of 100 µM ATP (Sigma), and 0.2 µl of 10 µCi/µl of [γ-32P]ATP.
(PerkinElmer Life Sciences) in kinase assay buffer was added to the beads. Beads were incubated for 30 min at 30 °C with shaking. The reaction was terminated by the addition of LDS sample buffer (Invitrogen) with 10% β-mercaptoethanol and heated at 75 °C for 10 min. Supernatants were resolved on a 10% Bis-Tris gel. The gel was dried for 1 h at 80 °C and exposed to x-ray film.

Co-immunoprecipitation—Myc-Aurora A was immunoprecipitated from 0.5 mg of 293T cell extracts at 4 °C using 20 μl of c-Myc-agarose conjugate slurry (clone 9E10, Santa Cruz) after preclearing with protein A/G-agarose beads (Santa Cruz) for 1 h with rotation. Immunoprecipitation was carried out for 2 h with rotation. Beads were washed extensively with lysis buffer plus protease and phosphatase inhibitors. Beads were resuspended in 4× sample buffer (Invitrogen) with 10% β-mercaptoethanol, and samples were heated at 75 °C for 10 min. Supernatants were resolved by SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Millipore). The membrane was blocked in 5% nonfat milk in Tris-buffered saline (TBS) plus 0.05% Tween 20 (TBS-T) and probed with primary antibody in 5% milk, TBS-T overnight at 4 °C (α-TPX2, Biologic and α-Aurora A, Cell Signaling Technologies). Membranes were incubated with goat α-mouse or α-rabbit-HRP (Santa Cruz) at 20 ng/ml for 1 h at room temperature, and immunodetection was carried out using enhanced chemiluminescence reagent (Amersham Biosciences).

Immunofluorescence Microscopy—HeLa cells were seeded onto 13-mm glass coverslips at 25,000 cells/well in 6-well plates, simultaneous with lentiviral transduction, and incubated for 96 h. Cells were washed with PBS and fixed with 4% paraformaldehyde in PBS for 45 min. Cells were rinsed with PBS, then immunofluorescence buffer (1% bovine serum albumin, 2% fetal bovine serum) and permeabilized in 0.5% Triton X-100 in PBS for 10 min. After PBS and immunofluorescence buffer rinses, coverslips were incubated with primary antibody (α-Myc, 9E10, 0.25/2.5 μg/ml) in a moist chamber for 45 min. Coverslips were washed extensively in PBS and incubated with AlexaFluor 555-conjugated goat anti-mouse IgG1, (Invitrogen) at 2 μg/ml for 40 min. After PBS washes, nuclei were counterstained with 1.43 μM 4′,6-diamidino-2-phenylindole (Invitrogen), mounted in Vectashield (Vector laboratories), and sealed onto slides. All steps were performed at room temperature.

Tumor DNA Sequencing—Exons 4 (S155R and V174M) and 8 (S361*) of the Aurora A gene were amplified from genomic DNA extracted from paraffin-embedded colon tumors using GoTaq polymerase (Promega) and the forward primers (5′-3′) GCTGGAGATGCATTTGAGTG (for exon 4), GACCTC-CAAAAAGAATACCG (for exon 8) and reverse primers (5′-3′) GGAGTTGGACTGTCTTCTCTGTC (for exon 4) and AGACTGTGTTGCTAGCTGATTG (for exon 8). A portion of the PCR reaction was directly sequenced in one or both directions using BigDye1.1 reagent (Applied Biosystems). Sequencing was performed by automated dideoxy method and analyzed using DNAStar Lasergene software.

Crystallization and Structure Determination—Hexagonal bipyramidal crystals of dephosphorylated Aurora A(S155R) were grown at 18 °C by vapor diffusion using 17% (w/v) PEG3350 and 15% (v/v) glycerol as well as buffer and sitting drops comprising a 1:1 mix of 14.5 mg/ml protein premixed with 10 mM ADP. Diffraction data were collected at 92 K and processed using Mosflm (35, 36). The structure was solved by molecular replacement using Phaser (36) with the coordinates of Aurora A(D274N) (PDB code 1OL6) as a search model. Phenix (37) was used for refinement, and Coot (38) was used for model building (residues 127–275, 291–301, and 307–388 are included in the final model). The statistics for data collection and refinement are shown in Table 2. Structure figures were prepared using PyMol (39).

RESULTS

Altered Biochemical Properties and Localization of Cancer-associated Aurora A Mutants—As the somatic mutations are located in the kinase domain (Fig. 1a), we hypothesized that they might affect catalytic activity. To characterize the activity of the Aurora A mutants relative to wild type, we transiently expressed them as Myc epitope-tagged fusion proteins in 293T cells. Equivalent amounts of each mutant protein were immunoprecipitated, and their kinase activities were determined by measuring phosphorylation of MBP, a generic kinase substrate. The Aurora A(V174M) mutant showed increased kinase activity relative to wild-type, whereas the Aurora A(S361*) mutation abolished activity (Fig. 1b). Interestingly, the Aurora A(S155R) mutant retains its kinase activity, although this is reduced compared with the wild type. To compare the intrinsic activity of the catalytic domain with or without the effects of the regulatory factor TPX2, we also measured the kinase activity of the wild-type and mutant kinase domains using recombinant proteins expressed and purified from E. coli (Fig. 1c). In this assay the activity of the wild-type Aurora A was increased in a concentration-dependent manner up to 7-fold by TPX2, as has been described previously (27). Aurora A(V174M) activity was 3-fold higher than wild-type Aurora A and was increased by TPX2, and at the maximum TPX2 concentration tested, the activity was 1.3-fold higher than wild type. Aurora A(S155R) showed slightly reduced kinase activity relative to wild type, and the difference in relative activity increased upon TPX2 addition from 1.5- to 3.3-fold. Thus, the relative kinase activities of the recombinant proteins in the presence of TPX2 supported the findings from the human cell immunoprecipitation kinase assay. These results suggest that the Aurora A(V174M) mutant constitutively increases the activity of the kinase, whereas the Aurora A(S155R) mutant has reduced activity due to loss of activation by TPX2.

We next determined the localization of the Aurora A mutants. Previous studies have shown that Aurora A localizes to the centrosome and to spindle microtubules (3). To examine localization of the Aurora A mutants, HeLa cells were infected with lentiviral constructs co-expressing GFP and Myc-tagged wild-type Aurora A, Aurora A(V174M), Aurora A(S155R), Aurora A(S361*), and empty vector, and Aurora A localization was visualized in GFP-positive cells using an α-Myc antibody. We classified the localization of each Aurora A variant in at least 25 cells, which revealed striking differences in localization (Fig. 1d, Table 1, supplemental Fig. S1). Wild-type and Aurora A(V174M) were localized to spindle microtubules and centrosomes in the characteristic “umbrella-like” pattern (Fig. 1d). By contrast, Aurora A(S155R) protein showed distinct localization.
A Mislocalized and Misregulated Aurora A Mutant

We investigated the effect of the mutations on the ability of TPX2 to bind Aurora A by coprecipitation using *Escherichia coli*-expressed proteins. Aurora A(V174M) bound TPX2 similarly to wild-type, but there was no observed interaction between Aurora A(S155R) and TPX2 (Fig. 2a). As a control, a mutant TPX2 (A12D/F16D) was unable to bind any of the Aurora A proteins. Additionally, we confirmed the association of Aurora A mutants with TPX2 by co-immunoprecipitation experiments in human cells. 293T cells were transiently transfected with wild-type Aurora A, Aurora A(V174M), Aurora A(S155R), Aurora A(S361*), and empty vector. Aurora A(V174M) bound TPX2 similarly to wild-type, whereas Aurora A(S155R) and Aurora A(S361*) showed no binding to TPX2 (Fig. 2b). Loss of TPX2 binding would explain the significantly reduced relative activity and mislocalization of Aurora A(S155R) that we observed in human tissue culture cells.

We expected that TPX2 would not be able to protect Aurora A(S155R) from dephosphorylation by PP1 because Aurora A(S155R) shows reduced binding to TPX2. To

to the centrosomes but not to the spindle microtubules. This pattern of localization is reminiscent of that observed when TPX2 protein is depleted by small interfering RNA (26). The truncated kinase inactive mutant Aurora A(S361*) showed a wider cytoplasmic staining rather than the typical centrosomal localization (Fig. 1d). The most likely explanation for the loss of activity and mislocalization is that the protein is misfolded due to the disruption of the $\alpha$H helix and loss of the $\alpha$I helix caused by the premature C-terminal truncation.

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PP1 negatively regulates the Aurora kinases, and the activation of Aurora A by TPX2 is at least partly due to the protection of phospho-Thr-288 (24, 25, 27, 40) from PP1-mediated dephosphorylation. We expected that TPX2 would not be able to protect Aurora A(S155R) from dephosphorylation by PP1 because Aurora A(S155R) shows reduced binding to TPX2. To
investigate this possibility, we carried out in vitro dephosphorylation assays using wild-type and mutant kinase domains recombinantly expressed in E. coli (Fig. 2c). Using this assay, we confirmed that Aurora A(V174M), like the wild-type protein, is completely dephosphorylated on Thr-288 by PP1. Wild-type and Aurora A(V174M) were protected from dephosphorylation by TPX2, but strikingly, Aurora A(S155R) was not dephosphorylated either in the presence or absence of TPX2. Therefore, although Aurora A(S155R) does not bind TPX2, it is nonetheless somewhat resistant to dephosphorylation by PP1 in vitro. This may indicate that the regulated inhibition of PP1 dephosphorylation by TPX2 is abrogated in Aurora A(S155R).

The S155R mutation was originally identified in a microsatellite instability-positive colon tumor (MIN+ /H11001 sample (33). To further investigate the occurrence of the somatic S155R Aurora A mutation in this tumor type, DNA samples from 40 MIN+ colon tumors and 20 microsatellite instability-negative colon tumors were analyzed. We did not find the V174M or S361* mutations in any of the samples, although this was not surprising because these mutations were originally identified in different tumor types. One sample from the MIN+ colon tumors with the Aurora A(S155R) mutation was found and was confirmed to be a somatic mutation as wild-type Aurora A was found in the plasma DNA from the same patient (Fig. 2d).

**Structural Basis of Aurora A(S155R)-altered Properties**—To understand the structural basis for the altered properties of Aurora A(S155R), we determined the structure to 2.5 Å resolution by x-ray crystallography (Table 2). Aurora A(S155R) superposes very closely onto the wild-type Aurora A structure with an overall Cα root mean square deviation of 0.7 Å for equivalent residues (Fig. 3a). The unphosphorylated activation loop is disordered as would be expected. Residue 155 lies in a loop between the second and third β-strands (β2-β3 loop, Figs. 1a and 3a), and Arg-155 and surrounding residues are well defined by clear electron density (Fig. 3b). There are several side-chain changes as well as a shift in the main chain in this region (Cα root mean square deviation for residues 153–157 was in the range of 0.9–1.7 Å; Fig. 3b, and supplemental Fig.
A Misllocated and Misregulated Aurora A Mutant

Notably, the conformation of the side chain of Arg-155 is stabilized by the formation of a salt bridge with Glu-152 and is packed between the side chains of Phe-157 and Trp-128 (Fig. 3b). Additionally, residue Phe-157 adopts a changed rotamer when compared with the WT Aurora A structure and packs against the hydrophobic portion of the Arg-155 side chain. When superposed on the WT Aurora A–TPX2 complex structure, it is clear that the side chains of Arg-155 and Phe-157 extend into the TPX2 binding site and would prevent the binding of TPX2 to Aurora A(S155R) (Fig. 3, c, d, and e). The structure of Aurora A(S155R) provides a clear rationale for loss of TPX2 binding through steric blocking.

The other main structural changes with respect to previous nucleotide-bound Aurora A structures are around the active site, including movements of the Gly-rich loop and C-helix and disorder in Glu-181 and the DFG motif (supplemental Fig. S2b). By contrast, in previously solved structures of Aurora A bound to nucleotide, Glu-181 forms a catalytically important salt bridge with Lys-161, and the DFG motif adopts an ordered, “in” position. The significance of these structural alterations and how they could be caused by the mutations is not clear. In addition to the ADP molecule bound to the active site, there was clear electron density for a second molecule of ADP forming a crystal packing contact between two Aurora A(S155R) molecules (supplemental Fig. S2, c and d). The structural changes in the vicinity of the S155R mutation reveal a cryptic binding site for a small, flat, hydrophobic molecule. In the structure, the site is filled with electron density which we have tentatively modeled as a third ADP molecule, although only the adenine moiety is well ordered (Figs. 3d and supplemental Fig 2e). This cryptic binding site would not occur in the wild-type protein because packing of the phenyl ring against the side chain of Ser-155 is unfavorable, and indeed nothing similar has been described in any of the previous Aurora A structures that resulted from crystallization in the presence of flat, hydrophobic molecules such as nucleotides or kinase inhibitors.

DISCUSSION

In tumors, the most common change involving Aurora A is mRNA overexpression with or without gene amplification. In the present report we characterize Aurora A somatic cancer mutations on a functional and structural level. Aurora A(V174M) and Aurora A(S155R) were found to be active, although with different levels of activity compared with wild type, whereas the activity of Aurora A(S361*) was abolished. The Aurora A(S361*) mutant was found in a lung cancer sample, a type of cancer in which abnormality of the

FIGURE 3. S155R mutation introduces an ordered, localized alteration in the structure of Aurora A. a, shown is superposition of Aurora A(S155R) (yellow) and TPX2/wild-type (WT) Aurora A (purple/cyan). The region around the mutation is indicated by a gray box. b, 2Fo − Fc electron density map contoured at 1.0σ in the vicinity of residue 155 is shown. c, superposition of Aurora A(S155R) and TPX2/WT Aurora A in the vicinity of residue 155 shows the steric clash between Arg-155/Phe-157 and the TPX2 binding site around F19. d, structure of Aurora A(S155R) around the binding site for a small, flat, hydrophobic molecule, modeled as the adenine of ADP is shown. e, the structure of TPX2/WT Aurora A is shown in the same view as c and d.

TABLE 2
Table of crystallographic and refinement statistics

| Crystals |
|----------|
| Space group | P4₁2₁2₁ |
| Lattice constants |
| a (Å) | 85.57 |
| b (Å) | 85.57 |
| c (Å) | 79.95 |

| Data collection |
|-----------------|
| X-ray source | Diamond I03 |
| Resolution range (Å), (highest resolution shell) | 85.4-2.5 (2.75-2.5) |
| Unique reflections | 242,938 |
| Completeness (%) | 99.1 (99.1) |
| Multiplicity | 7.5 (7.5) |
| Rmerge (%) | 7.3 (42.7) |
| I/σ(I) | 8.3 (1.8) |

| Refinement |
|------------|
| Number of residues | 261 |
| Number of waters | 30 |
| Rfactor (%) | 20.40 |
| Rfree (%) | 25.06 |

| Ramachandran plot |
|-------------------|
| Most favored (%) | 92.4 |
| Allowed (%) | 7.6 |
| Generously allowed (%) | 0 |
| Forbidden (%) | 0 |

*Free Rfactor was computed using 5% of the data assigned randomly.
$p53$ gene is one of the most common events (41). Thus, this inactive mutation might be considered to produce a similar effect to the frequent deletion or down-regulation of Aurora A observed in human breast cancer cell lines and in primary human tumors with low levels of $p53$ and also in $p53$ null mice (42). Aurora A(V174M) is more active than wild-type Aurora A; therefore, this mutation might be considered to produce a functional equivalent to Aurora A mRNA overexpression. Modeling of Aurora A(V174M) suggests that the mutation stabilizes the active conformation of the activation loop, thus increasing the activity of the kinase and reducing the requirement of TPX2 activation (supplemental Fig. S3, a and b). Interestingly, Aurora A(V174M) was identified in a melanoma sample, a tumor type in which $p53$ mutations are less frequent than in other tumors. These observations are consistent with a pattern in which increased Aurora A activity is negatively correlated with the frequency of $p53$ mutations, although much more research is needed to confirm and fully explain such a pattern.

The most dramatic change in function associated with any of the somatic mutations is the absence of binding of the Aurora A(S155R) mutant to TPX2, resulting in reduced kinase activity and mislocalization away from mitotic spindle microtubules. In addition, the efficiency of PP1 dephosphorylation is reduced in the Aurora A(S155R) mutant, and thus, its activity is somewhat decoupled from regulation. The effects of the S155R mutation on the interaction with TPX2 may be interpreted with confidence because the structure of the Aurora A-TPX2 complex is known. By contrast, additional studies are needed to further characterize the biochemical and structural interaction of Aurora A and PP1 so that the reduced efficiency of PP1 dephosphorylation of Aurora A(S155R) can be explained. The Aurora A(S155R) mutation alters the activity and regulation of the kinase, but how could this contribute to the development or survival of a cancerous cell? Studies in human cells and Caenorhabditis elegans in which mutants of TPX2 or TPX-L that did not bind Aurora A replaced the endogenous protein showed that elimination of the interaction resulted in defective mitotic spindles (43, 44). In the absence of the interaction with TPX2, Aurora A is localized exclusively to the centrosomes (43, 44). Human cells lacking the Aurora A-TPX2 interaction through engineered mutations in TPX2 exhibited decreased fidelity of cell division that may result in aneuploidy (44). Because Aurora A(S155R) is defective in its association with TPX2, this mutation may be selected during oncogenesis as it may produce errors in cell division. The altered regulation of Aurora A(S155R) by PP1 may also be significant because the phosphorylation and, hence, activity of this mutant could be sustained outside mitosis. Indeed, the loss of regulation by TPX2 and PP1 may synergistically contribute to cancer formation because these two regulators are key determinants of the phosphorylation state of Aurora A (25, 40). Several questions remain regarding the cancer relevance of these mutations. First, there is only indirect evidence that the three mutants play any role in cancer. Second, the V174M and S361* have only been identified as somatic mutations in single samples and, therefore, require further validation in the appropriate tissues. Third, as yet none of the mutants have been shown to promote tumorigenesis or cancer cell survival. Further studies will be required to discover whether these mutations do play a role in cancer and, if so, to dissect the mechanisms of their contributions.

The identification of cancer-associated mutations in human kinases has been and continues to be an important step toward the deduction of cancer development mechanisms and furthermore identifies rational targets for therapeutic intervention. Prime examples of driver mutations are those that promote increased kinase activity uncoupled from regulation. These mutations are found in conserved regulatory elements such as the DFG motif, Gly-rich loop, and activation loop, and they may be predicted as driver mutations with high confidence (45). Such mutations may also occur with high frequency (e.g. V600EBRAF) and are clear targets for therapeutic intervention using kinase inhibitors. The way that mutations in other locations contribute to cancer is likely to be kinase-specific, such as the loss of TPX2 binding in Aurora A(S155R), and will require individual investigation into each mutation.

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