Requirements for Aurora-A in Tissue Regeneration and Tumor Development in Adult Mammals

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Abstract

Aurora-A is a kinase involved in the formation and maturation of the mitotic spindle and chromosome segregation. This kinase is frequently overexpressed in human cancer, and its activity may confer resistance to antitumoral drugs such as Taxol. Inhibition of Aurora-A results in mitotic defects, and this kinase is considered as an attractive therapeutic target for cancer. Nevertheless, the specific requirements for this kinase in adult mammalian tissues remain unclear. Conditional genetic ablation of Aurora-A in adult tissues results in polyplid cells that display a DNA-damage–like response characterized by the upregulation of p53 and the cell-cycle inhibitor p21<sub>Cip1</sub>. This is accompanied by apoptotic, differentiation, or senescence markers in a tissue-specific manner. Therapeutic elimination of Aurora-A prevents the progression of skin and mammary gland tumors. However, this is not due to significant levels of apoptosis or senescence, but because Aurora-A-deficient tumors accumulate polyplid cells with limited proliferative potential. Thus, Aurora-A is required for tumor formation in vivo, and the differential response observed in various tissues might have relevant implications in current therapeutic strategies aimed at inhibiting this kinase in the treatment of human cancer. Cancer Res; 73(22): 6804–15. © 2013 AACR.

Introduction

Aurora kinases play critical roles in centrosome biology and chromosome segregation (1). The founding member of this protein family, Aurora-A, is primarily implicated in centrosome maturation and spindle assembly (2). Indeed, Aurora-A localizes to the centrosomes during interphase, and to both spindle poles and spindle microtubules during early mitosis. The best-known Aurora-A regulator is TPX2. Binding with TPX2 induces a conformational change in Aurora-A in such a way that the phosphorylated activation domain of this kinase adopts a more compact position, providing a better substrate-binding platform and hiding the activating phosphoryl group from the activity of phosphatase PP1 (3). Aurora-A has been documented to be involved in p53 regulation because it phosphorylates p53 at Ser315, leading to its Mdm2-mediated ubiquitination and subsequent proteolysis (4). Other functions of Aurora-A include the regulation of the translation of RNAs that contain cytoplasmic polyadenylation elements (CPE) at their 3′ untranslated region, which has been shown in the regulation of meiosis (5), α-CamKII synthesis at synapses (6), and the regulation of the cell-cycle regulators cyclin B1 and Cdk1 (7).

Interfering with Aurora-A expression or activity via siRNA expression, immunodepletion, or by specific inhibitors induces mitotic alterations that impair cell-cycle progression. In fact, its disruption in <i>Drosophila melanogaster</i>, <i>Caenorhabditis elegans</i>, and <i>Xenopus</i> causes defects in centrosome maturation and spindle formation (8–13). In addition, RNA interference (RNAi)-mediated reduction of Aurora-A expression in mammalian cell lines has been associated with abnormalities in mitotic entry, bipolar spindle formation, and mitotic progression (14–18). Moreover, Aurora-A is necessary for proper mitotic progression during mouse embryonic development, and its genetic ablation results in lethality at the morula stage (19–21).

Aurora-A is overexpressed in multiple tumor types, including breast, pancreatic, ovarian, and gastric carcinomas (22). In addition, Aurora-A has been included in the top 70 list of genes of the cancer-associated chromosome instability signature (23). Several small-molecule inhibitors have been shown to block its function, and their in vivo antitumor activity is currently under evaluation in clinical trials for the treatment of human cancer (24). Treatment with Aurora-A inhibitors has resulted in some limited but promising responses in early-phase clinical trials (25). Improving future trials will require...
Genetic Ablation of Aurora-A in Tumors

Further understanding of the physiologic effects of inhibiting Aurora-A in adult tissues. Despite all the reported data on the role of Aurora-A in lower organisms and in cultured cells, the requirements for this kinase in adult mammals and the cellular consequences of its elimination remain unclear. We have generated a mouse model that allows the conditional elimination of Aurora-A upon the activation of the Cre recombinase by 4-hydroxytamoxifen (4-OHT), the active metabolite of tamoxifen. Here, we show that ubiquitous ablation of Aurora-A in vivo is associated with an age-related phenotype characterized by a significant alteration of the proliferative tissues. Aurora-A-depleted tissues are characterized by a significant increase in mitotic and DNA damage markers, and with the presence of tetraploid or aneuploid cells, eventually resulting in impaired proliferation and senescence.

Materials and Methods

Generation and characterization of Aurora-A–mutant mice

To generate Aurora-A conditional mouse models, we used mice carrying the Aurkalox/+ allele (19), the RERTert/+ allele (26), the K14-CreERT2transgene (27), and the MMTV-PyV7transgene (28). After the appropriate crosses, we obtained the experimental (Aurkalox/+; RERTert/+; Aurkaloxlox; TgK14-CreERT2+/+ and Aurkaloxlox; RERTert/+; MMTV-PyV7+/+), and control (Aurkaloxlox; RERTert/+; Aurkaloxlox; TgK14-CreERT2+/+ and Aurkaloxlox; RERTert/+; MMTV-PyV7+/+) mice used in this work. These animals were maintained in a mixed background (129/Sv, CD1, C57BL/6j, and FVB/N), and were genotyped as reported previously (19, 26). Mice were housed in the pathogen-free animal facility of the Centro Nacional de Investigaciones Oncológicas (Madrid) in accordance with the animal-care standards of the institution. These animals were observed on a daily basis, and sick mice were killed humanely in accordance with the Guidelines for Humane End Points for Animals used in biomedical research. All animal protocols were approved by the Instituto de Salud Carlos III Committee for Animal Care and Research. For Cre activation, mice were either fed with tamoxifen-supplemented food and tumor palpable tumors at 11 to 13 weeks of age. Since that moment, mice were fed with tamoxifen-supplemented food and tumor volumes scored weekly by microtomography using an eXplore Vista scanner (GE Healthcare). Tumor volume was calculated using the formula [sagittal dimension (mm) x cross dimension (mm)]/2.

Tumorigenic experiments

Skin carcinogenesis induced by 7,12-dimethylbenz(a)-anthracene (DMBA) and 12-O-tetradecanoylphorbol-13-acetate (TPA) was performed as previously reported (31). Tumors were measured weekly in two bisecting diameters by using a caliper. Mice developed multiple lesions that were taken together to calculate total tumor mass per mouse. Mammary gland tumors were obtained by crossing the Aurkaloxlox model with MMTV-PyV7+/+ transgenic mice (28). Females showed palpable tumors at 11 to 13 weeks of age. Since that moment, mice were fed with tamoxifen-supplemented food and tumor volumes scored weekly by microtomography using an eXplore Vista scanner (GE Healthcare). Tumor volume was calculated using the formula [sagittal dimension (mm) x cross dimension (mm)]/2.

Generation, culture, and characterization of mouse embryonic fibroblasts

Mouse embryonic fibroblasts (MEF) were generated from E13.5 embryos and cultured using standard protocols (32). To eliminate Aurora-A from Aurkaloxlox, RERTert/+ MEFs, we followed the synchronization scheme depicted in Supplementary Fig. S1. We added 4-OHT (100 nmol/L final concentration; HT-904, Sigma-Aldrich) or infected the cells with adenoviruses expressing Cre (supplied by the Iowa University). The same MEFs incubated with vehicle or infected with adenoviruses expressing FLP were used as controls, respectively. For immunofluorescence, cells were rinsed with PBS and fixed in 4% PFA-PBS for 7 minutes and, then, left in cold methanol overnight at 20 °C. After being blocked with 1% BSA for 1 hour, phospho-histone H3 (P-H3) was detected using a specific antibody from Millipore (05-806). Images were acquired using a Leica D3000 microscope or confocal ultraspectral microscope Leica TCS-SP5. For time-lapse imaging experiments, asynchronous, H2B-GFP-expressing cells were recorded (5-minute frames during 13 hours) using a DeltaVision RT imaging system (Applied Precision, LLC; IX70/71; Olympus) equipped with a charge-coupled device camera (CoolSNAP HQ; Roper Scientific). Colony-formation assays were performed in immortal Aurkaloxlox, RERTert/+ MEFs that stably

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expressed the viral oncoproteins E1A or 16E6. A total of 5,000 cells were plated in triplicate and were nontreated or treated with 4-OHT. Three weeks later, colonies were stained with Giemsa and counted.

Karyotyping and scoring of aneuploidy

For metaphase spreads, cells were hypotonically swollen in 40% full-medium, 60% tap water for 5.5 minutes. Hypotonic treatment was stopped by adding an equal volume of Carnoy solution (75% pure methanol, 25% glacial acetic acid), cells were then spun down, and fixed with Carnoy solution for 10 minutes. After fixation, cells are dropped from a 5-cm height onto glass slides previously treated with 45% of acetic acid. Slides were mounted with ProLong Gold anti-fade reagent with 40,6-diamidino-2-phenylindole (DAPI; Invitrogen), and images were acquired with a Leica D9000 microscope and an ×60 PlanApo N 1.42 N.A. objective. Chromosomes from 30 cells per genotype were counted. In addition, aneuploidy was determined by measuring nuclear volumes in both cells growing in culture and tissues. Interphasic nuclear volumes were calculated using the following algorithm: 4/3πr³, where r = Feret diameter/2. Feret diameter was calculated using ImageJ on images of DAPI-stained fibroblasts or H&E-stained tissues.

Statistical and imaging analyses

Statistical analyses were performed using the Student t, χ², or log-rank tests (GraphPad Prism 5). All data are shown as mean ± SD; probabilities of P < 0.05 were considered significant. Images were quantified using ImageJ (National Institutes of Health, Bethesda, Maryland).

Results

Genetic ablation of Aurora-A induces mitotic defects, aneuploidy, and senescence in vitro

Because the lack of Aurora-A is not compatible with mouse development (19–21), a conditional mutant was generated by crossing the Aurkalox/lox conditional allele (19) with the RERTert/+ allele expressing a 4-OHT-inducible Cre recombinase under the RNA polymerase II regulatory sequences (26). We first used Aurkalox/lox; RERTert/+ MEFs to eliminate Aurora-A upon the addition of 4-OHT. Aurora-null cultures were characterized by a decrease in Aurka protein levels and activity (Supplementary Fig. S1), and accumulated a high number of phospho-histone H3-positive cells, in correlation with a significant increase in the duration of mitosis (Fig. 1A and Supplementary Fig. S1). In agreement with previous reports (19–21), very few Aurka-null cells were able to normally segregate their DNA during mitosis (7% of the Aurkalox/lox vs. 97% of the control Aurkalox/lox cells, n = 88 and n = 143, respectively, P < 0.0001). These mutant cells mainly exited mitosis without chromosome segregation or with abnormal chromosome segregation in the presence of lagging chromosomes and chromosome bridges (41% and 50%, respectively, of Aurora-null cells entering mitosis, n = 88; Fig. 1A, Supplementary Fig. S2, and Supplementary Videos). This led to the accumulation of giant nuclei, characteristic of polyploid cells (Fig. 1B), as well as an increase in the aneuploid cell population (Fig. 1C). No differences were observed in the percentage of apoptotic cells in Aurkalox/lox; RERTert/+ MEFs treated or nontreated with 4-OHT throughout the 2-week observation period depicted in Supplementary Fig. S1 (data not shown). By contrast, the percentage of senescent cells was significantly increased among the Aurkalox/lox MEFs infected with Adeno-Cre when compared with the control ones infected with Adeno-EGFP (61.84 ± 5.83 vs. 14.59 ± 1.97, P < 0.0001; Fig. 1D). We next engineered Aurkalox/lox; RERTert/ert MEFs to stably express oncoproteins E1A and 16E6, which inactivate the retinoblastoma protein (pRb) or p53, respectively. These two different cell lines were equally sensitive to the lack of Aurora-A when subjected to a clonogenic assay (Fig. 1E), suggesting that the proliferative defects induced by Aurora-A loss are independent of p53 and pRb function.

Conditional ablation of Aurora-A in adult mice

To analyze the consequences of Aurora-A ablation in an adult organism, Aurkalox/lox; RERTert/ert or Aurkalox/lox; RERTert/+ mice were fed with tamoxifen at 1 to 2 month of age for a minimum period of time of 4 weeks. As shown in Fig. 2A, a partial although significant reduction of the conditional alleles (Aurkalox/lox in favor of the deleted ones (Aurkalox/lox) was observed in Aurkalox/lox; RERTert/+ tissues after 4 weeks of treatment. This was associated with a significant reduction of the expression of Aurora-A protein in different tissues (Fig. 2B–D). Histopathologic studies of Aurora-A–deficient (hereafter, Aurkalox/lox) mice did not show major abnormalities in organs with low proliferative ratio such as the kidney, heart, pancreas, and brain (data not shown). However, tissues with high proliferation rates showed marked defects. We observed significant testicular atrophy, thinner and immature, epidermis as well as atrophy of the intestine, thymus, and spleen (Fig. 2C; Supplementary Fig. S3). Tissues from Aurkalox/lox mice showed seminiferous tubules with reduced quantity of germinal cells and lack of mature spermatozoa. Clear-cell depletion with reduced organ size were found in both red and white pulp in the spleen of Aurkalox/lox mice, with the red pulp being the most affected by a significant reduction in the number of cells. In the skin, although control mice showed hair follicles reaching the panicular cutaneous muscle, Aurkalox/lox hair follicles were mainly at the catagen–telogen stage, therefore, located entirely within the dermis. The intestine showed decreased crypts and villi length with abnormal representations of normal cell populations.

Aurkalox/lox mice showed external phenotypes, such as abnormal curvature of the upper spine and loss of hair—hallmarks typically associated with an age-related phenotype (Fig. 3A). Another common feature of the Aurkalox/lox mice was a reduction in their body weight. This reduction was observed as early as 1 week after the start of the tamoxifen treatment and, 3 weeks later, it reached a 30% of the body weight (Fig. 3B). These defects were not observed in control mice (Aurkalox/lox; RERTert/+ mice treated with tamoxifen; hereafter, Aurkalox/lox mice), which showed a slight (8%) and transient reduction in body weight within the first week (Fig. 3B). Importantly, Aurora-A ablation compromised the survival of adult mice because 40% of treated animals died during the 40 weeks following the tamoxifen treatment whereas all control mice survived in the presence of tamoxifen (Fig. 3C).
Histopathologic analysis of sick mice showed a pleiotropic phenotype preventing us from establishing a single common cause of death in all the cases. Several features found in Aurora-A null mice, such as thinner and immature epidermis, and atrophy of intestine, thymus, and spleen, could well be associated with death as a result of starvation, infection, dehydration, and/or multiorgan failure. Because the abnormal curvature of the upper spine, a condition called kyphosis, found in *Aurka*ΔΔ mice, could appear as a result of osteoporosis, we decided to analyze the bone density of *Aurka*ΔΔ; *RERTert* mice treated with tamoxifen for a long period of time. As it is shown in Fig. 3D, the densitometer images from *Aurka*ΔΔ; *RERTert* mice treated with tamoxifen for 4 months displayed a 10% decrease in their bone density when compared with control mice (0.0524 ± 0.0004 vs. 0.058 ± 0.0012 g/cm², P < 0.01). In addition, blood cells and a number of hematopoietic parameters were significantly reduced in the Aurora-A-depleted mice (Table 1). The reduction was significant in the case of lymphocytes, granulocytes, erythrocytes, platelets, hemoglobin, and the hematocrit.

Cellular defects associated with Aurora-A loss in vivo

In the absence of Aurora-A, several proliferative tissues such as the skin, spleen, and thymus showed a significant reduction in the expression of the proliferation marker Ki67 (Fig. 4A, Supplementary Fig. S4). In addition, this pattern was observed...
in other tissues characterized by low proliferation rates, such as the lung, liver, kidney, heart, and pancreas (Supplementary Fig. S4). A detailed analysis of one of the proliferative tissues (spleen) allowed us to visualize among the abnormalities detected in spleen, skin, and testis of Aurora-null mice. Scale bars, 100 μm. Right, Aurora-A expression is significantly diminished upon tamoxifen treatment in Aurora+/lox; RERTert/ert mice. Aurora-A immunohistochemical detection is shown for three representative proliferative tissues. Bar graphs indicate the quantification of the number of Aurora-A-positive cells (n = 3 mice per genotype; *, P < 0.05; **, P < 0.01). Scale bars, 100 μm.

**Aurora-A ablation in tissue regeneration and tumor growth**

To investigate the consequences of Aurora-A ablation in tissue regeneration in vivo, we made use of the hair follicle cycle. Aurora+/lox; RERTert/ert and Aurora+/lox; RERTert/ert mice were depilated on 2-cm² patches of their back-skin and treated with tamoxifen for 5 days. Seven days later, although Aurora−/− control mice were able to repopulate the back-skin with new hair, this was not the case with the Aurora+/lox mice (Fig. 5A). Follicular hairs in the Aurora-A null mice arrested at the last stage of the hair follicle cycle (telogene) and showed bigger nuclei (Fig. 5A), suggesting that Aurora-A–depleted cells were able to duplicate their genome but could not divide.

To further understand whether the lack of Aurora-A affects activation and/or differentiation of hair follicle stem cells, we studied the presence of labeling-retaining cells at the hair follicles. Aurora+/lox; RERTert/ert mice were injected with bromodeoxyuridine (BrdUrd) at P14 (when the first synchronized
hair cycle ends) to label their stem cells. Just before the second hair cycle was completed, mice were depilated on their backs and treated topically (back skin and tail) with either dimethyl sulfoxide (DMSO) or tamoxifen. Two weeks later, hair growth and treated topically (back skin and tail) with either dimethyl sulfoxide (DMSO) or tamoxifen. Two weeks later, hair growth

Cell proliferation was assessed by BrdUrd incorporation in tumor sections. BrdUrd-positive cells were significantly fewer in Aurora-A deficient tumors (Fig. 5B), suggesting the loss of Aurora-A results in growth arrest. To further confirm this, we depleted Aurora-A in tumor cells by treatment with tamoxifen during the indicated times. B, Aurora-A deficient mice treated with tamoxifen (Δ/Δ) show a significant loss of weight when compared with control (+/+) mice (P < 0.0001, n = 3). C, Aurora-A deficient mice also show a significantly different survival (log-rank, Mantel-Cox test) when compared with control mice (0.0524 < P < 0.01). D, Aurora-A deficient mice treated with tamoxifen (Δ/Δ) show a significant reduction in the bone density of Aurora-A deficient mice when compared with control mice (0.058 ± 0.0012, n = 3, g/cm², P < 0.01).

Table 1. Aurora-A-deficient mice are defective in several hematologic parameters

<table>
<thead>
<tr>
<th>Hematologic parameter</th>
<th>Aurora-A+/+ (n = 8)</th>
<th>Aurora-AΔ/Δ (n = 8)</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>P</th>
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<td>WBC (×10⁹/L)</td>
<td>9.82 ± 1.34</td>
<td>4.86 ± 0.98</td>
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<td>LYM (×10⁹/L)</td>
<td>7.23 ± 1.07</td>
<td>3.75 ± 0.82</td>
<td>*</td>
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<td>MID (×10⁹/L)</td>
<td>0.41 ± 0.08</td>
<td>0.27 ± 0.82</td>
<td>0.11</td>
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<tr>
<td>GRA (×10⁹/L)</td>
<td>2.18 ± 0.65</td>
<td>0.84 ± 0.27</td>
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<td>RBC (×10¹²/L)</td>
<td>8.68 ± 0.24</td>
<td>5.15 ± 0.73</td>
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<tr>
<td>HGB (g/dL)</td>
<td>13.31 ± 0.59</td>
<td>8.21 ± 1.36</td>
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<td>HCT (%)</td>
<td>40.20 ± 2.21</td>
<td>24.14 ± 2.95</td>
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<td>MCV (fl)</td>
<td>46.00 ± 2.09</td>
<td>48.88 ± 2.84</td>
<td>0.21</td>
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<tr>
<td>MCH (pg)</td>
<td>15.33 ± 0.59</td>
<td>15.80 ± 0.82</td>
<td>0.32</td>
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<td>MCHC (g/dL)</td>
<td>33.56 ± 1.60</td>
<td>32.84 ± 1.67</td>
<td>0.38</td>
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<td>RDWc (%)</td>
<td>18.21 ± 0.75</td>
<td>21.79 ± 1.88</td>
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<td>PLT (×10⁹/L)</td>
<td>741.3 ± 119.20</td>
<td>297.60 ± 110.50</td>
<td>0.50</td>
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<td>PCT (%)</td>
<td>0.45 ± 0.11</td>
<td>0.24 ± 0.09</td>
<td>0.08</td>
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<td>MPV (fl)</td>
<td>5.87 ± 0.15</td>
<td>7.40 ± 0.69</td>
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<td>PDWc</td>
<td>32.15 ± 0.53</td>
<td>37.26 ± 1.57</td>
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NOTE: ** P < 0.05; * P < 0.01; * * * P < 0.001.

Abbreviations: GRA, granulocytes count; HCT, hematocrit; HGB, hemoglobin; LYM, lymphocyte count; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MID, monocytes/eosinophils count; MPV, mean platelet volume; PCT, platelet percentage; PDWc, platelet distribution width; PLT, platelet count; RBC, red blood cell count; RDWc, red cell distribution width; WBC, total white blood cell count.

Genetic Ablation of Aurora-A in Tumors
A and lack of proliferation (low Ki67 levels; Fig. 6B). It is important to note that no differences were found in the expression of p53 (data not shown) nor in the levels of apoptotic (Fig. 6B) or senescent cells (data not shown). Therefore, the arrest observed in skin tumors treated with tamoxifen is not p53-dependent or due to an induction of apoptosis or senescence. Interestingly, Aurora-A–null tissues display increased levels of differentiation markers, such as loricrin or...
involucrin (Supplementary Fig. S6), in agreement with previous reports suggesting that the block of mitosis results in increased differentiation in the skin (33).

Furthermore, we analyzed the effect of Aurora-A deletion in more aggressive tumors induced by the expression of the polyomavirus middle T (PyVT) oncogene in mammary glands (28). We generated Aurora−/lox; RERTert/ert and Aurora−/lox; RERTert/ert females that also expressed the MMTV-PyVT transgene. As soon as they had palpable tumors (at ~11–13 weeks of age), mice were treated with tamoxifen to induce the deletion of the Aurora-A gene. A clear increase in the size of multifocal adenocarcinomas was observed in control females (Aurka+/+) whereas Aurora−/− tumors grew at a significantly slower rate (Fig. 6C; tumor size 10 weeks after tamoxifen addition of 4,173 ± 1,380 among the Aurora−/− tumors, vs. 1,129 ± 599 among the Aurora−/−/− tumors; n = 4, P < 0.05). Furthermore, secondary metastatic tumors in the lung characteristic of this model were significantly reduced among Aurora−/−/− mice (P < 0.05; Fig. 6D). These Aurora−/−/− tumors were characterized by a significant reduction of the proliferative markers (Ki67) without an increase in the apoptotic (C3A) or senescent (SA-βgal–associated activity) markers (Supplementary Fig. S7). However, Aurora−/− tumors were less active metabolically (PET data in Fig. 6E). Similar to Aurora−/− skin tumors, the Aurora−/− mammary gland tumors were characterized by increased levels of differentiation markers (p63 and cK8; Supplementary Fig. S6). In addition, these mutant
Figure 6. Aurora-A ablation results in arrest of skin and mammary gland tumors in vivo. A, skin tumors were induced in Aurkalox/lox; TgK14CreERT2/lox mice using a DMBA + TPA treatment. The resulting papillomas were treated topically with tamoxifen or carrier (DMSO). Graph shows tumor volumes relative to the pretreatment size (mean ± SEM; *, P < 0.05). B, panels show representative pictures of skin tumor sections in which expression of Aurora-A, Ki67, and caspase-3 (Ca3) is detected. Scale bar, 100 μm. C, depletion of Aurora-A also inhibited the growth of polyomavirus middle T (PyVT) oncogene-induced mammary tumors. Tumor growth was compared in Aurka+/lox and Aurkalox/lox; RERTert/ert females that also expressed the MMTV-PyVT transgene. At 11 to 13 weeks of age, mice were treated with tamoxifen, and tumor size was measured every 2 weeks by computed tomography. Graph represents the longitudinal data obtained for seven mice of each group. At time 0, tamoxifen was administered to all the experimental mice. The experiment was completed 10 weeks later. Significant differences were detected in the tumor volumes at 8 and 10 weeks post tamoxifen treatment (*, P < 0.05; n = 4–12 per group in each time point). D, lung metastases were also reduced in Aurora-A null mice (Δ/Δ) when compared with controls (+/Δ). Bar graph shows the quantification of the metastases identified in 10 random fields from three lung sections (*, P < 0.05; n = 5 mice per group). Representative lungs are shown in pictures on the right. Scale bar, 2 mm. E, Δ/Δ tumors did not increase their metabolic activity with time. In 2 weeks (between week 1 and 3 of tamoxifen treatment), tumors significantly increased their metabolic activity, measured by PET (upper bar graph). However, Aurora-A null, Δ/Δ, tumors did not change their metabolic status (***, P < 0.001; n = 10). H, heart; B, bladder. F, nucleus volume in cells from mammary tumors is significantly larger in Δ/Δ samples than in controls. Tumor cells from mammary tumors were grown in culture for a week and, after DAPI staining, a minimum of 23 nuclei were quantified per sample. Bar graph shows nucleus volume of tumor cells from three mice of each type (*, P < 0.05 two-way ANOVA test). Images are representative DAPI-stained nuclei from Δ/Δ and +/-/Δ tumors. Scale bar, 10 μm.
tumors contained giant nuclei and mitotic abnormalities rarely observed in Aurora−/− tumors (Fig. 6F and Supplementary Fig. S2). A similar significant increase in the nuclear volume of Aurora−/− tumoral cells was observed in the skin adenocarcinomas induced by TPA plus DMBA treatment (Supplementary Fig. S7). All these data suggest that the lack of Aurora-A impairs cell division and induces a polyploid phenotype incompatible with cell proliferation, whereas apoptosis and senescence are rare events associated with Aurora-A elimination in these tumors.

Discussion

Aurora-A is required for cellular proliferation in cultured cells, and its genetic inactivation results in early embryonic lethality in the mouse and severe defects in skin development (14, 15, 18–21, 34, 35). Due to its critical role in the cell cycle, several Aurora-A inhibitors are currently studied as antitumoral agents (24, 25). Because many of these inhibitors can act in a similar way against the three Aurora family members and also be active against other kinases, it is important to discriminate the specific effects of the inhibition of Aurora-A associated with the use of these inhibitors. Using a conditional knockout model, we show here that Aurora-A is critical for continued cell proliferation in adult animals. Importantly, this phenotype is not compensated by the other two Aurora family members, Aurora-B and Aurora-C, which are highly similar but quite different from Aurora-A (36). In fact, these two groups of Aurora kinases display different subcellular localization and bind to different partners (INCENP/survivin/borealin in the case of Aurora-B/C and TPX2/Bora in the case of Aurora-A). Interestingly, a single amino acid difference allows TPX2 to discriminate between Aurora-A and -B (37), and an Aurora-A mutant for that residue rescues Aurora-B loss of function (38, 39). Our results confirm that, despite the similarity and common origin of mammalian Aurora kinases, these proteins display specific characteristics that limit their possible compensatory roles.

Because Aurora-A inhibitors are not specific for tumor cells, our results provide critical genetic data to separate the relevance of this kinase in vivo and as a cancer target. We have added significant information on the effect of Aurora-A dosage in tumor development and progression. It had been previously shown that overexpression or heterozygous expression of Aurora-A induces malignant transformation (20, 40, 41). Here, we show that its complete loss prevents tumor formation and inhibits the progression of chemical or genetically induced tumorigenesis. As this apparent paradox is common to other mitotic regulators, one possibility is that both partial loss- or gain-of-expression induces chromosomal instability and favors tumorigenesis. On the other hand, as all these proteins are required for the cell cycle, their complete depletion impairs cell proliferation, a feature that has been critical for their consideration as putative cancer targets (25, 42). Our results in vitro and in vivo suggest that lack of Aurora-A results in defective chromosome segregation and the generation of tetraploid or aneuploid cells. This is accompanied by a stress response characterized by DNA damage and the induction of p53 and p21Cip1. Aneuploid cells are frequently characterized by the induction of a DNA damage response as a consequence of a higher frequency of DNA damage and/or the induction of replicative stress (43). Moreover, abnormal and/or prolonged mitosis has been shown to induce accumulation of DNA breaks (44). More recently, Aurora-A has been identified in a genome-wide siRNA screen as one of the genes whose abrogation led to elevated levels of H2AX phosphorylation (45). This data is of special relevance in tumorigenesis, because it implies that the inhibition of Aurora-A could sensitize tumors to anticancer agents that work better against cancer cells with high levels of DNA damage.

Some Aurora-A inhibitors such as MLN8054 and MLN8237 (Milenium) induce senescence in myeloma and colon cell lines as well as in lung and melanoma xenografts (46–48). We have observed a significant induction of senescence in Aurora-A–depleted spleens. However, neither senescence nor apoptosis seem to be a frequent observation in other cases wherein Aurora-A is eliminated from normal or cancer cells. Nevertheless, our data suggest that Aurora-A inactivation seems to be sufficient to arrest tumor growth in the absence of both apoptosis and senescence (Fig. 6). Cell-cycle arrest caused by Aurora-A deficiency is also independent on p53 or Rb function (Fig. 1). However, Aurora-A–defective tissues show a clear induction of p53 (Fig. 4). The upregulation of p53 might be due to DNA damage induced upon Aurora-A depletion and/or could be a consequence of an increase in its stability, as it has been previously reported to occur when Aurora-A is silenced (4). However, depletion of p53 does not rescue the lack of proliferation of Aurora-A–null mouse fibroblasts (Fig. 1). Our data suggest that proliferative defects caused by Aurora-A deficiency are mostly a consequence of defective DNA synthesis in polyploid or aneuploid cells. Thus, although Aurora-A ablation allows DNA replication in cells with low ploidy, these cells finally arrest in a G0-like state with high ploidy. According to this hypothesis, Tsunematsu and colleagues have recently demonstrated that Aurora-A controls DNA replication by stabilizing Geminin in mitosis, which in turn inhibits SCF^Nedd2-mediated degradation of Cdt1 and ensures prereplicative complex formation in the subsequent cell cycle (49). Therefore, the lack of proliferation induced by Aurora-A depletion could be a consequence of a defective DNA replication rather than a p53-induced cell-cycle arrest. In addition, we have observed an intriguing induction of differentiation upon mitotic failure (Supplementary Fig. S6), an observation that may explain tumor arrest in the absence of apoptosis or senescence, at least in some specific tissues. Further understanding of these pathways will have important implications in future antitumor therapies based on the use of inhibitors of Aurora-A or other mitotic targets (25). Given the lack of proper biomarkers for testing the effect of Aurora-A inhibition in clinical trials, a quantification of ploidy or nuclear size in treated tumors could be of help in future assays.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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