Aurora-A controls cancer cell radio- and chemoresistance via ATM/Chk2-mediated DNA repair networks

Huizheng Sun a,b,1, Yan Wang a,b,1, Ziliang Wang a,b, Jiao Meng a,b, Zihao Qi a,b, Gong Yang a,b,c,*

Abstract

High expression of Aurora kinase A (Aurora-A) has been found to confer cancer cell radio- and chemoresistance, however, the underlying mechanism is unclear. In this study, by using Aurora-A cDNA/shRNA or the specific inhibitor VX680, we show that Aurora-A upregulates cell proliferation, cell cycle progression, and anchorage-independent growth to enhance cell resistance to cisplatin and X-ray irradiation through dysregulation of DNA damage repair networks. Mechanistic studies showed that Aurora-A promoted the expression of ATM/Chk2, but suppressed the expression of BRCA1/2, ATR/Chk1, p53, p53S (Ser15), H2AX, γH2AX (Ser319), and RAD51. Aurora-A inhibited the focus formation of γH2AX in response to ionizing irradiation. Treatment of cells overexpressing Aurora-A and ATM/Chk2 with the ATM specific inhibitor KU-55933 increased the cell sensitivity to cisplatin and irradiation through increasing the phosphorylation of p53 at Ser15 and inhibiting the expression of Chk2, γH2AX (Ser319), and RAD51. Further study revealed that BRCA1/2 counteracted the function of Aurora-A to suppress the expression of ATM/Chk2, but to activate the expression of ATR/Chk1, pp53, γH2AX, and RAD51, leading to the enhanced cell sensitivity to irradiation and cisplatin, which was also supported by the results from animal assays. Thus, our data provide strong evidences that Aurora-A and BRCA1/2 inversely control the sensitivity of cancer cells to radio- and chemotherapy through the ATM/Chk2-mediated DNA repair networks, indicating that the DNA repair molecules including ATM/Chk2 may be considered for the targeted therapy against cancers with overexpression of Aurora-A.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Radio- and chemotherapy are two principal approaches mostly used to destroy cancer cells through inducing irreparable DNA damage [1]. However, the acquired resistance of cancer cells to ionization radiation (IR) or chemotherapy is the major obstacle to improving cancer patient survival. The serine/threonine kinase Aurora-A, namely Aurora kinase A (AURKA), breast tumor activated kinase (BTAK), or serine threonine kinase 15 (STK15), is a member of the Aurora kinase family reported to induce centrosome amplification, chromosomal instability and transformation in mammalian cells [2]. However, the function of Aurora-A has not been fully explored in cancer cells. Over the past several years, increasing evidences have shown that overexpression of Aurora-A is associated with radio- and chemoresistance. For instances, the amplification of Aurora-A increases radioresistance in laryngeal cancer cells [3], and Aurora-A may lead to cervical cancer radiosresistance through enhancing the transcription activity of NF-κB [4]. In esophageal squamous cell carcinoma (ESCC) cells, overexpression of Aurora-A inhibits the cisplatin- or UV irradiation-induced apoptosis, but silencing of the endogenous Aurora-A kinase with siRNA substantially enhances the sensitivity to cisplatin or UV [5]. In patients with epithelial ovarian cancer, overexpression of Aurora-A is correlated with the resistance to carboplatin and indicates a poor prognosis [6]. Silencing of Aurora-A increases the colorectal cancer stem cell sensitivity to 5-FU and oxaliplatin [7]. The abnormal expression of Aurora-A is involved in chemoresistance through ZNF217 in breast cancer cells [8]. In a recent study, Aurora-A was proven to play a critical role in the acquired chemoresistance of chronic myelogenous leukemia cells to the tyrosine kinase inhibitor ima-tinib [9]. But the mechanism that Aurora-A induces radio- and chemoresistance is not clear.

ATM-Chk2 (Ataxia telangiectasia mutated kinase/the checkpoint kinase 2) and ATR-Chk1 (Ataxia telangiectasia and Rad3-related protein/the checkpoint kinase 1) are two major branches at the upstream of the DNA damage repair signaling [10], and the active ATM usually phosphorylates the histone H2AX at Ser139, yielding γH2AX to trigger DSB repair [11]. Breast cancer type 1/2 susceptibility proteins (BRCA1/2), two tumor suppressors, also function to participate in DNA repair and cancer cell resistance.

Abbreviations: Aur-A, Aurora-A cDNA-infected cells; Aur-Ai, Aurora-A shRNA-infected cells; BRCA1i, BRCA1 shRNA-infected cells; BRCA2i, BRCA2 shRNA-infected cells; Scr, scrambled shRNA-infected cells; IR, Irradiation; Cis, Cisplatin; KU, KU-55933, the inhibitor of ATM

* Corresponding author at: Cancer Institute, Fudan University Shanghai Cancer Center, Department of Oncology, Shanghai Medical College, Fudan University, 270 Dong’an Road, Shanghai 200032, China.

† H. Sun and Y. Wang equally contributed to this work.
Atmosphere of 5% CO2 and 95% air. The mounting evidences have suggested that Aurora-A may regulate BRCA1/2 and the other DNA repair proteins. Aurora-A physically binds to and phosphorylates BRCA1 at Ser308, leading to the abrogation of G2/M checkpoint [15]. In ovarian cancer cells, Aurora-A represses BRCA2 expression, while silencing of Aurora-A restores the level of DNA repair foci of both BRCA2 and Rad51 after γ-irradiation [16]. Soursise et al. reported that, in normal mammary cells, overexpression of Aurora-A modulates the activity of the checkpoint kinase 1 (Chk1) and inhibits RAD51 recruitment to DNA double-strand-breaks (DSBs) induced by X-ray irradiation [17]. They also found that the decreased DSBR sensitivity causes cancer cells to PARP inhibition, which requires the inhibition of Chk1 by the Polo-like kinase 1 (PLK1), and that in pancreatic cancer cells, ectopic expression of Aurora-A inhibits the homologous recombination (HR) in cells with the wide-type BRCA2, but has no such effect in cells with the mutant BRCA2 [17].

In the present study, we used breast, pancreatic, and ovarian cancer cells to investigate the function of Aurora-A in radio- and chemoresistance. Our results indicate that Aurora-A and BRCA1/2 are mutually suppressed to control cell sensitivity to radio- and chemotherapy mainly through the ATM/Chk2-mediated DNA repair networks associated with p53, pp35, γH2AX, and RAD51.

2. Materials and methods

2.1. Cell lines and cell culture

Human breast cancer cell lines (MCF-7 and MDA-MB-231), pancreatic cancer cell lines (OVCA420 and OVCA429), and retroviral packaging cells (Phoenix amphotropic cells) were purchased from American Type Culture Collection (ATCC, US). MCF-7, PANC-1 and Phoenix cells (Phoenix amphotropic cells) were purchased from American Type Culture Collection (ATCC, US). MDA-MB-231, BXPC3, OVCA420 and OVCA429 were main-
were recorded as optical density (OD) absorbance at 570 nm subtracted from the background OD at 490 nm. IC50 was calculated by the algebraic formula of the improved Karber method \( \text{lgIC50} = \frac{X_m - I}{P - (P_m - P_n)/4} \); \( X_m \): lg (maximum dose), \( I \): lg (maximum dose / adjacent dose), \( P \): sum of the positive reaction rates, \( P_m \): maximum positive reaction rate, \( P_n \): minimum positive reaction rate). The experiment was repeated three times in triplicate.

2.6. Cell cycle and apoptosis analysis

Cell cycle and apoptosis were analyzed through flow cytometry (FAC Station; BD Biosciences, US) using the previously described protocols [18]. Propidium iodide and RNase were purchased from Sigma-Aldrich (Missouri, US); Annexin V-fluorescence apoptosis detection kit was acquired from BD Biosciences PharMingen. The assay was repeated three times.

2.7. Immunoblotting analysis

To analyze protein expression in cells, immunoblotting was performed as previously described [18]. Antibodies against the following proteins were obtained from Cell Signaling Technology (Massachusetts, US): Aurora-A, H2AX, ATM, Chk1, Chk2, pp53 (Ser15). Antibodies against the following proteins were obtained from Santa Cruz Technology (California, US): p53, BRCA1, BRCA2, RAD51. The antibodies against γH2AX (Ser139) and β-actin were purchased from Biolegend and Sigma-Aldrich companies, respectively. The secondary antibodies were F(ab)2 fragment of donkey anti-mouse immunoglobulin or of donkey anti-rabbit immunoglobulin linked to horseradish peroxidase from Amersham Biosciences (Buckinghamshire, UK). Immunoblot reagents were from an electrochemiluminescence kit (Amersham Biosciences). The intensity of protein bands was quantified with Image J software downloaded from NIH website (http://imagej.nih.gov/ij/).

2.8. Immunofluorescence

Immunofluorescence staining was performed according to a published protocol [16]. DNA dye DAPI and primary antibodies against Aurora-A and γH2AX (Ser139) were obtained from Molecular Probes, Cell Signaling Technology and Biolegend. The secondary antibodies...
were either FITC- or Texas red-conjugated donkey F(ab)2 fragments against mouse IgG or rabbit IgG (Jackson ImmunoResearch Laboratory). For DNA damage foci examination, cells were first treated with X-ray irradiation (8 Gy), then incubated for 6 h before immunofluorescence staining. All stained cells were examined and photographed with a Leica SP5 confocal fluorescence microscope.

2.9. Xenograft tumors in nude mice

To detect the in vivo effects of Aurora-A on chemo- and radioresistance, we selected the series of MDA-MB-231 cells to generate xenograft mouse tumor model. Briefly, $5 \times 10^6$ cells of MDA-MB-231/Scr, MDA-MB-231/vector, MDA-MB-231/Aur-Ai, MDA-MB-231/BRCA1,
MDA-MB-231/BRC2 cells were subcutaneously injected into 4- to 6-week-old BALB/c athymic nude mice (Department of Laboratory Animals, Fudan University). The animal experiments were approved by the Institutional Animal Care and Use Committee of Fudan University and performed following Institutional Guidelines and Protocols. Each cell line was bilaterally injected into 18 mice, for a total of 36 injections. The longest diameter “a” and the shortest diameter “b” of tumors were measured and the tumor volume was calculated with the use of the following formula: tumor volume (in mm$^3$) = $a \times b^2 \times 0.52$ [17], where 0.52 is a constant to calculate the volume of an ellipsoid. When the volume of tumors reached 1.0 cm in diameter (around 38 days post injection), the mice injected with each cell line were randomly divided into 3 subgroups: control group, X-ray group and cisplatin group, 6 mice for each subgroup. The mice in X-ray group were exposed to 10 Gy X-ray once every 6 days for a total of three exposures, while the mice in cisplatin group were injected intraperitoneally with 0.05 mg cisplatin per gram of body weight once every 6 days for a total of three times. The control groups were either irradiated with 0 Gy (for MDA-MB-231/vector cells) or treated with DMSO (for MDA-MB-231/Scr cells). When the biggest tumor in control and diluent groups reached 2.0 cm in diameter, all experimented mice were sacrificed simultaneously and the tumor size was measured accordingly.

2.10. Statistical analysis

The Student $t$ test was used for statistical analysis of all data. $P < 0.05$ was considered significant.

3. Results

3.1. Aurora-A promotes the cell proliferation, cell cycle progression and anchorage-independent growth

To investigate the roles of Aurora-A in radio- and chemoresistance, we delivered cDNA or shRNA of Aurora-A into breast, pancreatic, and ovarian cancer cell lines that were pretested with low or high expression of Aurora-A, and first characterized the proliferation, cell cycle progression, and anchorage independent growth of resulting cell lines. The results from Western blotting showed that Aurora-A was overexpressed...
bands was analyzed by Image J software and labeled under each band, and the intensity-based graph is shown in SFig. 7A. Overexpression of Aurora-A promoted cell proliferation, but knockdown of Aurora-A restrained cell growth (Fig. 1).

Compared with the control cells, the cell population in S phase was increased by 7.8%, 5.5%, and 4.4%, but that in G0–G1 phase was decreased by 1.7%, 7.2%, and 10.9%, respectively in MCF-7/Aur-A, PANC-1/Aur-A, and OVCA420/Aur-A cells. The number of cells in G2–M phase was inconsistently altered. Silencing of Aurora-A in MDA-MB-231, BXPC3, and OVCA429 cells reduced the number of S phase cells by 4.5%, 39.2%, and 3.8%, but increased the number of cells in G0–G1 phase by 23.2%, 13.7%, and 6.2%, respectively. The population in G2–M phase was decreased by 18.8% and 2.3% in MDA-MB-231/Aur-Ai and OVCA429/Aur-Ai, but was increased in BXPC3/Aur-Ai (Fig. 1B). These data suggest that Aurora-A functions to stimulate overall cell cycle progression mainly through G1–S transition, which is consistent with our recent report tested in different cell lines [19].

The results from soft agar assay showed that the number of colonies was twice more in PANC-1/Aur-A cells than in vector cells, and the colonies formed by BXPC3/Aur-Ai cells were much fewer than those by BXPC3/Scr cells. Similarly, compared with the corresponding control cells, the number of colonies was increased by 45.2% and 68.6% in MCF-7/Aur-A and OVCA420/Aur-A, but was decreased by 39.1% and 25.3% in MDA-MB-231/Aur-Ai and OVCA429/Aur-Ai (Fig. 1C).

The above results suggested that the amplification of Aurora-A promoted the cancer cell proliferation, cell cycle progression, and in vitro tumorigenicity.

3.2. Overexpression of Aurora-A confers cancer cell resistance to X-ray irradiation and cisplatin treatment

To evaluate the effect of Aurora-A on cell sensitivity to ionizing radiation (IR), cells were exposed to X-ray (8 Gy) and detected for early and late apoptosis by flow cytometry. As shown in Fig. 2 and SFig. 2A, in response to irradiation, the proportion of early apoptotic cells with double staining of PI and FITC is indicated in the fourth quadrant, while the early apoptosis with single staining of FITC is indicated in the fourth quadrant. B. Quantitative analysis of cell apoptosis from five repeated tests by flow cytometry. C. Changes of DNA damage repair associated proteins in cells treated with IR or cisplatin. The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in SFig. 7B. D. The effects of ATM inhibition on DNA damage repair proteins tested by Western blot, β-actin was used as a loading control. The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in SFig. 7E.

Next, we tested whether Aurora-A was associated with chemoresistance. As shown in Fig. 2E, compared with the corresponding control cells, the half maximal inhibitory concentration (IC50) of cisplatin was increased by 4.4%, 12.4%, and 4.4% in MCF-7/Aur-A, PANC-1/Aur-A, and OVCA420/Aur-A cells, respectively. The number of early apoptotic cells was reduced by 1.3%, 4.7% and 0.1%, respectively, in MCF-7/Aur-A, PANC-1/Aur-A, and OVCA420/Aur-A cells, while the late apoptotic cells were decreased by 6.5%, 8.5% and 9.0%, respectively, in cells overexpressing Aurora-A (Fig. 2F–G and SFig. 2B).

Since VX680, a small compound, was reported to selectively inhibit the activity of Aurora kinase, we used the chemical at the concentration (0.6 nM) to specifically inhibit the activity of Aurora-A as reported elsewhere [20], to test whether the inactivation of Aurora-A by VX680 was able to increase cell apoptosis to chemotherapeutic treatment. Concurrent treatment of MCF-7/Aur-A, PANC-1/Aur-A, and OVCA420/Aur-A cells with VX680 and cisplatin induced the number of both
early and late apoptotic cells more than did the treatment with cisplatin alone (Fig. 2F–G and Sfig. 3 upper panel).

On the other hand, silencing of Aurora-A decreased the IC50s and increased apoptosis in MDA-MB-231/Aur-Ai, BXPC3/Aur-Ai, and OVCA429/Aur-Ai cells, compared with the control cells (Fig. 2H–J and Sfig. 3 lower panel). These results demonstrated that overexpression of Aurora-A inhibited the apoptosis induced by cisplatin.

3.3. Aurora-A enhances ATM/Chk2 to dysregulate the downstream DNA damage repair molecules

It is well-known that the essential mechanism to kill cancer cells by chemotherapeutic compounds or IR is to induce cell apoptosis through irreparable DNA damage. Because ATM/Chk2 and ATR/Chk1 function at the upstream of signal pathways to participate in DNA repair, we first detected the expression of ATM/Chk2 and ATR/Chk1 in cells transfected with Aurora-A cDNA or shRNA by immunoblotting. Compared with that in control cells, overexpression or silencing of Aurora-A elevated or reduced the expression of ATM and Chk2, but suppressed or increased ATR (except in BXPC3 cells) and Chk1 expression (Fig. 3A). The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in Sfig. 3B.

We further tested other downstream DNA repair molecules and found that the expression of ATR, Chk1, p53, pp53 (Ser15), H2AX, γH2AX, and RAD51 was suppressed by overexpression of Aurora-A, but was increased by knockdown of Aurora-A except that ATR was decreased in BXPC3/Aur-Ai cells, compared with the control cells (Fig. 3B). The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in Sfig. 3C.

γH2AX is involved in the recruitment of DNA damage repair factors to the sites of damaged DNA, including DSBs [21].

---

Fig. 5. Expression of BRCA1/2 and the effects of BRCA1/2 on cell cycle progression, anchorage-independent colony formation. A. Detection of BRCA1 and BRCA2 in cells expressing Aurora-A cDNA or shRNA. B. Analysis of BRCA1/2 and Aurora-A after cells were treated with BRCA1/2 shRNAs or cDNAs. β-actin was used as a loading control. The intensity of protein bands is labeled under each band, and the intensity-based graphs are shown in Sfig. 8A–C. C. Quantitative analysis of cell cycle distribution. Cells stained with propidium iodide were analyzed by flow cytometry. Data were collected from three independent assays. D. Anchorage independent growth in soft agar. The colony formation rate of control cell lines were set at 100%. *P < 0.05, **P < 0.01. Data from three independent experiments were analyzed. E. Xenograft tumor volumes burdened in mice injected with MDA-MB-231 and its derivatives. F–G. Alterations of xenograft tumor volumes in mice treated with IR or cisplatin after 38 days.*P < 0.05, **P < 0.01.
some other DNA lesions such as DNA single-stranded regions induced by ultraviolet C irradiation [22]. The localization of γH2AX foci after DNA damage most likely represents the repair efficiency of damaged DNA [22]. To validate the potential effect of Aurora-A on DNA repair, we examined the formation of γH2AX foci after IR and found a negative correlation between Aurora-A and γH2AX foci (Fig. 3C and SFig. 4). These results demonstrated that Aurora-A might enhance ATM/Chk2 to dysregulate the DNA damage repair networks associated with radio- and chemoresistance.

3.4 Inhibition of ATM increases the radio- and chemosensitivity in cells overexpressing Aurora-A

The above results indicated that Aurora-A might control radio- and chemoresistance through the upregulation of ATM/Chk2. To confirm this notion, we treated cells expressing high levels of Aurora-A and ATM/Chk2 with KU-55933, a potent, selective and ATP-competitive inhibitor of ATM kinase at the concentration of 20 nM.

As shown in Fig. 4, in response to irradiation, the treatment with KU-55933 increased the early and late apoptotic cells by 0.4% and 7.2% in MCF-7/Aur-A, by 7.1% and 1.3% in MDA-MB-231, respectively, compared with diluent treatment alone (Fig. 4A–B). Treatment with KU-55933 in addition to cisplatin increased the early and late apoptotic cells by 2.2% and 4.4% in MCF-7/Aur-A, by 24.6% and 5.5% in MDA-MB-231 cells compared with diluent and cisplatin treatment (Fig. 4A–B). These data suggested that the inhibition of ATM activity enhanced the sensitivity of cancer cells to irradiation and cisplatin.

To analyze the proteins associated with ATM-mediated DNA damage repair, we first treated breast and ovarian cancer cells overexpressing Aurora-A/ATM with X-ray (8 Gy) or cisplatin (10 mg/L) separately, and then treated these cells with the ATM inhibitor KU-55933 along with cisplatin or IR. As shown in Fig. 4C, the expression of pp53 (Ser15), Chk2, γH2AX (Ser139), and RAD51 were elevated or unchanged in response to DNA damage. Compared with control cells treated with DMSO, cells treated with KU-55933, or with KU-55933 + cisplatin, or with KU-55933 + IR reduced the expression of Chk2, but increased the phosphorylation of p53 (Ser15). Inhibition of ATM also reduced the levels of γH2AX (except in MDA-MB-231 cells treated with KU-55933 + cisplatin) and RAD51 (except in MDA-MB-231 cells treated with KU-55933 or KU-55933 + cisplatin, and OVCA420/Aur-A treated with KU-55933) (Fig. 4D), indicating a slight difference of DNA repair signaling between ATM and Aurora-A. The intensity of protein bands is labeled under each band, and the intensity-based graphs are shown in SFig. 7D–E.

These results demonstrated that the inhibition of ATM could diminish the Aurora-A-mediated radio- and chemoresistance through regulation of the downstream signal molecules in DNA repair pathways.

3.5 BRCA1/2 suppresses Aurora-A to reduce the cell proliferation, cell cycle progression, in vitro and in vivo tumorigenicity

Aurora-A has been reported to inhibit the expression of BRCA1/2 [15,16] and BRCA1/2 may inversely suppress the activity or expression of Aurora-A kinase [23,24]. Thus, BRCA1/2 may counteract the function of Aurora-A to elevate cancer cell sensitivity to treatment with radiation and genotoxic reagents. To validate this hypothesis, we first tested the expression of BRCA1/2 in cells either expressing Aurora-A cDNA or Aurora-A shRNA. As shown by Western blotting in Fig. 5A, compared with the control cells, the expression level of BRCA1/2 was down-regulated in Aurora-A-transfected cells (MCF-7/Aur-A, PAN-1/Aur-A, and OVCA420/Aur-A), but was elevated in Aurora-A-silenced cells (MDA-MB-231/Aur-Ai, BXPC3/Aur-Ai, and OVCA429/Aur-Ai). The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in SFig. 8A.

To further test the negative regulation between Aurora-A and BRCA1/2, shRNAs or cDNAs of BRCA1/2 were transfected into cells with high expression of BRCA1/2 or Aurora-A. Compared with the control cells, silencing of BRCA1/2 expression increased the expression of Aurora-A, while overexpression of BRCA1/2 reduced the expression of Aurora-A (Fig. 5B). The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in SFig. 8B–C.

Next, we examined cell proliferation, cell cycle progression, and in vitro tumorigenicity in cells transfected with BRCA1/2 cDNAs or shRNAs. Cell proliferation increased after BRCA1/2 was silenced, while overexpression of BRCA1/2 reduced the cell growth (Sfig. 5A). The data from cell cycle analysis showed that the knockdown of BRCA1 decreased the G0–G1 population, but increased the G2–M population in MCF-7/BRCA1i cells compared with MCF-7/Scr cells. No changes in cell cycle were observed in BRCA2-silenced MCF-7 cells compared with the control cells (Fig. 5C). However, in comparison with MDA-MB-231/vector cells, overexpression of BRCA1 or BRCA2 increased the number of cells by 29.9% and 29.0% at G0–G1 phase and reduced the number of cells by 4.6% and 0.7% at S phase and by 25.3% and 28.3% at G2–M phase in MDA-MB-231/BRCA1 and MDA-MB-231/BRCA2 cells, respectively (Fig. 5C).

The soft agar assay showed that silencing of BRCA1 or BRCA2 increased the number of colonies formed by MCF-7/BRCA1i and MCF-7/BRCA2i cells, while overexpression of BRCA1 or BRCA2 decreased the number of colonies formed by MDA-MB-231/BRCA1 and MDA-MB-231/BRCA2 cells (Fig. 5D and Sfig. 5B). Similar results were obtained from ovarian and pancreatic cancer cell lines (data not present). To strengthen the aforementioned results, we performed animal assays by using MDA-MB-231 cells and their derivatives. Mice burdened with tumors at 38 days post injection were treated with or without IR or cisplatin as described in MM section. We first found that overexpression of Aurora-A or knockdown of BRCA1 decreased tumor growth (Fig. 5E). Treatment of cells expressing Aur-Ai or BRCA1/2 with IR or cisplatin markedly reduced the tumor growth, compared with the tumors formed by cells expressing vector or Scr after the same treatments (Fig. 5F–G).

These results suggested that BRCA1/2 might counteract the function of Aurora-A to inversely regulate cell proliferation, cell cycle progression, in vitro and in vivo tumorigenesis in breast, pancreatic and ovarian cancer cells, which may subsequently attenuate the radio- and chemoresistance of cancer cells.

3.6 BRCA1/2 regulate ATM/Chk2 and DNA damage repair signaling to enhance the radio- and chemosensitivity

To test the effect of BRCA1/2 on DNA damage repair, we first detected the expression of the same molecules tested above. As shown in Fig. 6A, silencing of BRCA1 increased the expression of ATM and Chk2 (except in MCF-7/BRCA1i), but had no effect on RAD51, while silencing of BRCA2 decreased both ATM and RAD51 expression. However, the expression of p53, pp53 (Ser15) (except in MCF-7/BRCA1i and MCF-7/BRCA2i), and γH2AX was decreased after silencing of BRCA1 or BRCA2. Overexpression of BRCA1 decreased the expression of ATM (except in OVCA429/BRCA1), Chk2 (except in MDA-MB-231/BRCA1), but the effect on RAD51 was inconsistent. Overexpression of BRCA2 slightly increased ATM, and RAD51 (except in MDA-MB-231/BRCA2), but decreased Chk2, indicating a regulatory difference between BRCA1 and BRCA2. Overexpression of BRCA1/2 enhanced the expression of p53, pp53, and γH2AX. These data suggested that BRCA1/2 might control DNA damage response through ATM/Chk2. The intensity of protein bands is labeled under each band, and the intensity-based graph is shown in SFig. 8D.

To detect the radio- and chemosensitivity, cells were treated with X-ray (8 Gy) or cisplatin (10 mg/L). In response to IR, the number of early apoptotic cells was reduced only in MCF-7/BRCA1i cells,
but that of late apoptotic cells was reduced in both MCF-7/BRCT1i and MCF-7/BRCT2i cells, compared with MCF-7/Scr cells. The numbers of both early and late apoptotic cells were markedly increased in MDA-MB-231/BRCT1/2 cells compared with MDA-MB-231/vector cells (Fig. 6B–C, S5 Fig. A). In response to cisplatin, the early apoptotic cells were increased after BRCA1/2 was silenced in MCF-7 cells, but the late apoptotic cells were remarkably decreased. Both the early and late apoptotic cells were markedly increased after the ectopic expression of BRCA1/2 in MDA-MB-231 cells (Fig. 6D–E, S5 Fig. A). To strengthen the above results, we delivered BRCA1/2 cDNAs or shRNAs into OVCA420/Aur-A and OVCA429/Aur-Ai cells, respectively, and treated the resulting cells with X-ray and cisplatin. As shown in Fig. 6F–G and in SFig. 5B, in response to cisplatin, introduction of BRCA1/2 in OVCA420/Aur-A cells increased the drug sensitivity with decreased IC50 (≤20 mg/L), compared to control cells treated with vector or scrambled shRNA. Silencing of BRCA1/2 increased the drug resistance with increased IC50 (≥20 mg/L). Treatment of cells with IR increased or decreased apoptosis in cells transfected with BRCA1/2 cDNAs or BRCA1/2 shRNAs (data not shown). These results suggested that Aurora-A and BRCA1/2 might be mutually suppressed to modulate cancer cell response to radio- and chemotherapy through dysregulation of ATM/Chk2-mediated DNA repair networks.

4. Discussion

It is well-known that the resistance of cancer cells to radio- and chemotherapy is usually associated with the dysregulated DNA damage repair response. The effector proteins involved in the DNA damage response are either directly phosphorylated by ATM/ATR or by the checkpoint kinases Chk2 and Chk1 that act at the downstream of ATM and ATR [25]. A recent study revealed that Aurora-A modulates the activation of PLK1 to impair Chk1, resulting in the inhibition of RAD51 and the decreased HR [17], while the phosphorylation of p53 at serine 15 is associated with ATM in response to IR [26]. In this study, we performed a comprehensive study of Aurora-A in six cell lines from three different cancer types. We found, by both in vitro and in vivo experiments, that Aurora-A and BRCA1/2 inversely control cancer cell sensitivity to IR and cisplatin through the ATM/ Chk2-mediated DNA damage repair networks, although a few inconsistent results including those of cell cycle distribution were seen in different cell lines due to their genetic diversification. Our results showed that the overexpression of Aurora-A suppressed the expression of ATR, Chk1, p53, pp53 (Ser15), H2AX, γH2AX (Ser139), and RAD51, but enhances the levels of ATM and Chk2 in most of the cell lines (Fig. 3A–C). Furthermore, we showed that the inactivation of ATM by the specific inhibitor KU-55933 downregulated Chk2, but upregulated pp53 to reduce the radio- and chemoresistance (Fig. 4), indicating that the abnormal signaling of ATM/Chk2/p53 may play a critical role in Aurora-A-induced cancer cell radio- and chemoresistance, which is partially supported by the results from glioma cells [27].

H2AX can be phosphorylated as γH2AX by ATM and forms foci to be recruited for DNA repair along with RAD 51 upon DNA damage [28,29]. We showed that Aurora-A decreased the focus formation of γH2AX in response to IR, indicating that Aurora-A may dysregulate DNA repair through the γH2AX-mediated homologous recombination. We found that the expression of γH2AX and RAD51 was not increased in cells treated with KU-55933 alone or with KU-55933 plus cisplatin or IR compared with cells treated with DMSO. This may indicate that the downregulation of γH2AX and RAD51 by Aurora-A may be independent of ATM-Chk2 signaling and not essential to Aurora-A-induced radio- and chemoresistance, although detailed studies may be needed to validate this notion.

Studies have shown that BRCA1 acts in multiple aspects to repair the DSBs via HR [30], and is required for the phosphorylation of p53 (Ser15) and a G1/S arrest following IR-induced DNA damage [31]. BRCA2 is associated with the G1/S and the G2/M checkpoints [32] and also performs core functions in HR [33]. The BRC repeats of BRCA2 modulate the DNA-binding selectivity of RAD51, which coats ssDNA of the damaged DNA and initiates strand exchange between the paired DNA molecules [33]. In this study, we showed that the effects of Aurora-A on cell proliferation, cell cycle progression, anchorage-independent colony formation, and cancer cell resistance to IR and cisplatin treatment could be mimicked or reversed by the silencing or overexpression of BRCA1/2. Moreover, Aurora-A and BRCA1/2 inversely controlled the DNA damage responsive proteins including ATM/Chk2, ATR/Chk1, p53, RAD51, and the phosphorylation of p53 (Ser15), γH2AX (Ser139). Thus, based on the literature and our results, the cancer cell radio- and chemoresistance driven by Aurora-A may be caused by insufficient cell cycle checkpoint controls and deficient DNA damage repair networks that are counteractively managed by the functional BRCA1/2 molecules.

Aurora-A is known to regulate BRCA1 through phosphorylation [15], but whether it also negatively regulates BRCA2 through phosphorylation has not yet been reported. We have recently reported that Aurora-A and BRCA2 are mutually suppressed to control ovarian cancer tumorigenesis through regulation of RAS-associated genetic instability [19]. Overexpression of Aurora-A inhibits BRCA2 expression and induces malignant transformation of immortalized human ovarian surface epithelial cells, while silencing of Aurora-A in RAS-transformed cells enhances BRCA2 expression and reduces tumor growth [19]. Introduction of BRCA2 into RAS-transformed cells diminished Aurora-A expression and tumor growth [19]. Although co-localization of Aurora-A and BRCA2 has been found in the midbody of late mitotic cells during cytokinesis, the essential regulation between Aurora-A and BRCA2 has not been defined [19]. As others and we previously reported, both Aurora-A and BRCA2 could be regulated through proteolytic-mediated degradation [19,34,35]. Thus, we presumed that Aurora-A may regulate BRCA2 through phosphorylation and proteosome-mediated degradation, while BRCA1/2 may regulate Aurora-A expression through a feedback proteolysis-mediated degradation. Nevertheless, more investigations are needed to decode these speculations.

Taken together, our data suggest that Aurora-A controls radio- and chemoresistance through the ATM/Chk2-mediated dysregulation of DNA damage repair networks including pp53, γH2AX, and RAD51, whereas BRCA1/2 suppress Aurora-A to counteract the Aurora-A-induced radio- and chemoresistance by remodeling the DNA repair pathways. However, the molecular mechanism of how BRCA1/2 negatively regulate the expression of Aurora-A still needs further investigations although this notion has been evidenced or discussed in the current study as well as in our previous publications [16,24].

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamcr.2014.01.019.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (91129721 and 81372797), by the Shanghai Pujiang Program (11P1402200) from the Shanghai Municipal Government of China, and by the Doctoral Fund of Ministry of Education of China (20120071110079) for Gong Yang.