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

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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, pp53 (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.

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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 ionizing 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 radioresistance 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 imatinib [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

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

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repair [12]. In normal cells, the wild type BRCA1/2 and their partner proteins usually perform their functions to repair DNA lesions induced by IR or genotoxic agents, while in cancer cells, mutation or inactivation of BRCA1/2 often causes DNA repair deficiency, resulting in increased radio- and chemosensitivity. However, most of the recurrent or metastatic cancer cells are featured with the acquired resistance to treatment with IR and genotoxic agents due to the dysregulation of DNA repair networks associated with BRCA1/2 and ATM/Chk2 [13,14].

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 BRCA2, and increases the number of the DNA repair foci of both BRCA2 and Rad51 after γ-irradiation [16]. Sourisseau 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 DSB repair sensitizes 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, pp53, 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 (PANC-1 and BXPC3), ovarian epithelial 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 were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco). MDA-MB-231, BXPC3, OVCA420 and OVCA429 were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium. Both the cell culture media were supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 μg/ml). All cells were incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air.

2.2. Plasmid construction and cell transfection or viral infection

To enhance the expression of Aurora-A, BRCA1, and BRCA2, human wide type cDNAs of Aurora-A, BRCA1 and BRCA2 were inserted into pBabe/puromycin, pcDNA3.1-neomycin, or pCIN-neomycin, respectively. Viruses from pBabe vectors were produced and used to infect MCF-7, PANC-1 and OVCA420 cells, and to generate Aurora-A overexpression cell lines: MCF-7/Aur-A, PANC-1/Aur-A, and OVCA420/Aur-A using the previously published methods [16]. The control cell lines were generated by infection of the same cell lines with viruses containing empty vectors. pcDNA3.1 and pCIN vectors contain BRCA1 and BRCA2 cDNAs were transfected into MDA-MB-231, BXPC3 and OVCA429 by Fugene 6 (Roche) according to the manufacturer's instructions, to generate BRCA1 or BRCA2 overexpression cell lines: MDA-MB-231/BRCA1, MDA-MB-231/BRCA2, BXPC3/BRCA1, BXPC3/BRCA2, OVCA429/BRCA1, OVCA429/BRCA2, and OVCA420/Aur-A/BRCA1, OVCA420/Aur-A/BRCA2. Cells transfected with vectors were used as controls. The resulting cells were selected with puromycin (1.5–2.0 μg/ml) or neomycin (0.5–2.5 mg/ml) for 7–14 days.

To silence the expression of Aurora-A, BRCA1 and BRCA2, the DNA oligonucleotides used to generate shRNA against the open reading frame of mRNA were 5′-GUCUUGUGUCCUUCAAAUU-3′(Aurora-A shRNA), 5′-AAGUACGAGAUUUAGUCCG-3′ (BRCA1 shRNA) and 5′-ACAAUUACGAACCAAACCG-3′ (BRCA2 shRNA). pBabe/U6-puromycin-Aurora-Ai, pBabe/U6-neomycin-BRCA1i and pBabe/U6-neomycin-BRCA2i were generated according to the previously reported method [16]. The control vectors were similarly constructed by directly inserting a scrambled shRNA (Scr) into pBabe/U6-neomycin [16]. Retroviral particles were generated by using the same method mentioned above, and were used to infect and generate new cell lines including MDA-MB-231/Aur-Ai, BXPC3/Aur-Ai, and OVCA429/Aur-Ai cell lines, and MCF-7/BRCA1i, MCF-7/BRCA2i, PANC-1/BRCA1i, PANC-1/BRCA2i, OVCA420/BRCA1i, OVCA420/BRCA2i, and OVCA429/Aur-Ai/BRCA1i, OVCA429/Aur-Ai/BRCA2i. Corresponding control cells expressing scrambled shRNA were labeled as Scr. The infected cells were selected with puromycin (1.5–2.0 μg/ml) or neomycin (0.5–2.5 mg/ml) for 7–14 days.

2.3. Cell proliferation and anchorage-independent colony

To test cell proliferation, 5×10^3 cells (5×10^4 cells of BXPC3) were seeded into 12-well plates (each cell line in 15 wells) and incubated at 37 °C in an atmosphere of 5% CO_2 and 95% air and counted from 3 wells every 2 days individually for a total of 8 days (4 counts). The number of cells was recorded. The assay was repeated three times in duplicate.

For anchorage-independent colony formation, 5×10^3 cells of each cell line were suspended in 1 ml of medium with 0.35% agarose (Life Technologies, US), and the suspension was placed on top of 4 ml of solidified 0.7% agarose. Triplicate cultures of each cell type were maintained at 37 °C in a 5% CO_2 atmosphere, and the fresh medium was fed once a week. The number of colonies >50 μm (~100 cells, for MCF-7, PANC-1 and OVCA420 cell lines), or >30 μm (~50 cells, for MDA-MB-231, BXPC3 and OVCA429 cell lines) in diameter in each dish was counted at 14 to 21 days. The assay was repeated at least three times in duplicate.

2.4. Chemical compounds and X-ray irradiation

The Aurora kinase inhibitor VX680 and the ATM inhibitor KU-55933 were purchased from Selleck Company (Texas, US), and cisplatin were purchased from QiLu pharmaceutical company (Shandong, China) and dissolved in DMSO. The final concentration of VX680 was 0.6 nM, which specifically inhibits the activity of Aurora-A according to the manufacturer's instructions, while KU-55933 was 13 nM. The final concentration of cisplatin was determined for different cell lines and the experimental purpose. The final concentration of DMSO as diluent in cell culture used throughout the study did not exceed 0.1%.

For X-ray irradiation, $1–3 \times 10^6$ cells were seeded into 6-well plates and incubated at 37 °C. When the cell density reached 80%, the cells were exposed to X-ray irradiation at 8 Gy as pretested and harvested for apoptosis analysis 3 days later (4 days for OVCA420 and its derivatives).

2.5. The half-maximal inhibitory concentration

The half-maximal inhibitory concentration (IC50) was measured by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assay. Cells were seeded in 96-well plates at a density of 8000 cells/well in 100 μl of medium (DMEM/RPMI 1640). After overnight incubation at 37 °C, cisplatin at a series of concentrations was added to each well. After treatment with cisplatin for 48 h, 20 μl of 0.5 mg/ml MTT mixed with 180 μl medium was added to each well for incubation of 4 h, followed by 150 μl of DMSO for 10 min. Data

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