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Inhibition of AKT sensitizes chemoresistant ovarian cancer cells to cisplatin by abrogating S and G2/M arrest

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ABSTRACT

The phosphatidylinositol 3-kinase (PI3K)/Akt pathway is frequently altered in human malignancies and Akt over-expression and/or activation induces malignant transformation and chemoresistance. However, the role of Akt in the mechanisms of chemoresistance remains elusive. Here we reported that cisplatin treatment of chemosensitive, but not resistant, ovarian cancer cells (OVCAs) markedly increased the cell proportion in sub-G1 phase. Cisplatin however caused a significant accumulation of the resistant cells in S and G2/M phases, which was associated with a rapid and sustained checkpoint kinase 1 (Chk1) activation. In contrast, while cisplatin also elicited a rapid activation of Chk1 in sensitive cells, it markedly decreased total ChK1 and phospho-Chk1 contents over 12 h. Over-expression of dominant negative (DN)-AKT alone increased phospho-Chk1 content, and induced G2/M arrest and apoptosis. However, it inhibited Chk1 activation and G2/M arrest with combination of cisplatin treatment, resulting in p53-independent apoptosis. Furthermore, the responses of the chemoresistant cells to cisplatin were attenuated with forced expression of constitutive active AKT2. Chk1 knock-down also facilitated cisplatin-induced apoptosis in chemoresistant cells. Our studies implicate that, in addition to its cell survival and anti-apoptotic actions, Akt might also play an important role in the regulation of G2-M transition, possibly via up-regulation of Chk1 activity and stability. These data provide strong support for the concept that Akt is important in cell cycle regulation in the control of chemosensitivity in OVCAs and offers an alternate regulatory pathway for the development of rationale therapy for cisplatin-resistant ovarian cancer.

1. Introduction

Since the introduction into clinical trials, cisplatin (CDDP) has had a major impact in cancer medicine, changing the course of therapeutic management of several tumors. Despite high tumour response rates to platinum-based chemotherapy in ovarian cancer, the clinical success of cisplatin is compromised due to the emergence of drug resistance. Current understanding chemoresistance includes altered drug uptake, increased drug inactivation, evasion of apoptosis and enhanced ability to repair DNA damage (Fraser et al., 2003a, 2003b; Madhusudan and Hickson, 2005; Saczko et al., 2014). Accumulating evidence has demonstrated a role for the phosphatidylinositol 3-kinase (PI3K)/Akt pathway in the resistance to a number of anti-tumor agents via several mechanisms (Zhang et al., 2015; Ali et al., 2015, 2012; Fraser et al., 2008; Yang et al., 2006). We and others have demonstrated that cells expressing a constitutively active Akt2 renders ovarian carcer cells (OVCAs) resistant to cisplatin, an effect that could be reversed by the PI3K inhibitor, LY294002, suggesting that inhibition of the PI3K/Akt pathway could potentially act as a potent adjuvant to traditional chemotherapies (Ali et al., 2015; Fraser et al., 2008; Yang et al., 2006). Moreover, cisplatin treatment responsiveness has been correlated to molecular markers such as EGFR and AKT in several tumors (Jedlinski et al., 2013; Köberle et al., 2010; Shaw and Vanderhyden, 2007). Study on molecular mechanism of Akt conferring chemoresistance is mainly focused on regulation of several downstream targets in p53-independent and –dependent anti-apoptotic action. Since Akt activation plays a potent role in the genesis of cancer and alteration in cell-cycle progression and apoptotic processes are basic features of treated tumor cells, which is unlikely to be explained solely by the anti-apoptotic properties of this kinase.

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Actually, the cellular response to DNA damage involves cell cycle delays, increased repair, and apoptosis. Although many effective cancer therapies work by causing cell death due to DNA damage, resistance to these therapeutic agents remains a significant limitation in the treatment of cancer. One important mechanism of drug resistance has been attributed to cell cycle delays, also called checkpoints, which provide opportunities for cells to repair DNA damage (Madhusudan and Hickson, 2005). DNA damage causes cell cycle arrest in G1, S, or G2/M to prevent replication on damaged DNA or to prevent aberrant mitosis. The G1 arrest is dependent upon wild-type p53 activity, whereas S and G2 arrest do not require p53, so cells mutated for p53 (about 50% of tumors) arrest primarily in S or G2 in response to damage. Checkpoint kinase 1 (Chk1) is a central component of genome surveillance pathways and is a key regulator of the cell cycle and cell survival. Chk1 impacts various stages of the cell cycle including the S phase, G2/M transition and M phase (Zhang and Hunter, 2014). Chk1 also contributes to DNA repair processes. Chk1 primarily phosphorylates Cdc25 which results in its proteasomal degradation (Patil et al., 2013). The degradation has an inhibitory effect on the formation of cyclin-dependent kinase complexes, which are key drivers of the cell cycle (Liu et al., 2000). Through targeting Cdc25, cell cycle arrest can occur at multiple time points including the G1/S transition, S phase and G2/M transition (Zhang and Hunter, 2014).

In this study, we further investigated whether Akt also affected on cell sensitivity through influencing cell cycle checkpoints in responding to DNA damage, and whether Akt inhibition sensitized the chemoresistant OVCAs to cisplatin by regulating cell cycle and promoting apoptosis.

2. Materials and methods

2.1. Reagents

Cisplatin, Hoechst 33,258, phenylmethylsulfonyl fluoride (PMSF), sodium orthovanadate (Na_3VO4) and aprotinin were purchased from Sigma (St. Louis, MO). Rat monoclonal anti-HA was purchased from Roche (clone 3F10, Palo Alto, CA). Rabbit polyclonal anti-PARP antibodies were from Cell Signaling Technology (Beverly, CA). Mouse total chk1, rabbit phosphor-chk1 (S345), rabbit total and phosphor-cdc2 (Tyr 15), mouse polyclonal anti-phospho-histone H3 (Serine 10), Rabbit polyclonal anti phosphor-Histone H3 (Ser 10) antibody (Alexa Fluor® 488 conjugate) and rabbit polyclonal anti-caspase 3 antibodies were from Cell Signaling Technology.

Mouse anti-glyceraldehyde phosphate dehydrogenase (GAPDH) (ab8245) was from Abcam (Cambridge, UK). Small inhibitory RNA (siRNA) to Chk1 was purchased from Cell Signaling Technology. Control siRNA was from Dharmacon (Lafayette, CO). Ribojuice siRNA transfection reagent was from Novagen (San Diego, CA). Adenoviral construct containing HA-tagged, kinase-dead DN-Akt was a generous gift from Dr. Kenneth Walsh (Cardiovascular Research, St. Elizabeth's Medical Centre, Boston, MA). All adenovirus stock solutions were CsCl purified.

2.2. Cell culture

Cisplatin-sensitive (OV2008 and A2780s) and -resistant (C13* and A2780cp) cell lines and P53-null SKOV3 cells were cultured as reported previously (Du et al., 2013). Cells were plated at a density of 5×10^4 cells/ cm^2 on 6-well plates or 60-mm dishes 18 h prior to the initiation of treatment. At the time of treatment, cell density was <85%.

2.3. Creation of stably transfected cell lines

A2780s cells were stably transfected with pcDNA3 vector (Invitrogen) containing constitutively active HA-tagged, myristoylated Akt2 or pcDNA3 alone as reported previously in Fraser M*et al*(Fraser et al., 2003a, 2003b, 2008).

2.4. Adenovirus infection

All cells were infected with appropriate adenoviral constructs as reported in our previous report (Fraser et al., 2003a, 2003b, 2008). Infection with LacZ adenovirus was used to normalize the total concentration of adenovirus in each treatment group. Adenovirus infection efficiency was determined by western blotting detecting HA tag.

2.5. RNA interference

C13* or SKOV3 cells were transfected with 100 nmol/L Chk1 or negative control siRNA for 48 h. Cells were then treated with cisplatin and harvested for subsequent analysis as indicated.

2.6. Western blot analyses

Western blotting was done as previously described (Ali et al., 2015; Yang et al., 2006; Du et al., 2013). Membranes were incubated overnight at 4°C in primary antibodies (anti-HA, 1:20,000; anti-chk1 1:1000; anti- phosphor-chk1(S345) 1:1000; anti-cdc2 1:1000; anti-phosphor-cdc2 (Tyr 15) 1:1000; anti-caspase 3 1:1000; anti–PARP, 1:1000; anti-phosphor-Histone 3 (Ser 10) 1:1000 anti-GAPDH, 1:20,000), followed by horseradish peroxidase–conjugated anti-rabbit or anti-mouse or anti-rat secondary antibody (1:5000) incubation at room temperature for 1 h. Peroxidase activity was visualized with ECL kit (Amersham Biosciences, Piscataway, NJ). Results were scanned and analyzed using Scion Image software (Scion, Inc., Frederick, MD).

2.7. Propidium iodide staining and cell cycle analysis

Floating and adherent cell were collected and fixed in 70% ethanol overnight at −20°C. After washing with PBS, cell were incubated with propidium iodide (PI) staining solution (0.1% sodium citrate, 0.1% Triton X-100, 50 μg/ml PI (Sigma), 0.1 mg/ml DNase-free RNase A (Sigma) for 20 min at 37°C. Samples were analyzed on a Beckman Coulter FC500. Cells were analyzed on a Becton Dickinson fluorescence-activated cell sorter (FACScan) with Cell Quest Pro software (Becton Dickinson). In each sample, 20,000 events were counted. Data analysis was conducted with FlowJo software (Tree Star).

2.8. Assessment of apoptosis

After treatment, cells were harvested and the percentage of apoptosis was determined by Hoechst 33,248 staining as previously reported (Ali et al., 2015). Cells were counted with the counter "blinded" to sample identity to avoid experimental bias.

2.9. Statistical analyses

All results were given as mean ± SEM of at least three independent experiments. Data were analyzed by two-way ANOVA and Bonferroni posttest to test the differences between groups (PRISM software version 5.0, GraphPad, San Diego, CA). Statistical significance was inferred at $P < 0.05$.

3. Results

3.1. Cisplatin treatment induced cycle arrest at G1 phase in sensitive OVCAs but S and G2/M phase in resistant OVCAs

Consistent with our previous data, cisplatin treatment significantly induced apoptosis in sensitive cells (OV2008 and A2780s) and had no effect on the resistant variants (C13* and A2780cp) (Fig. 1A). To determine if cell cycle regulation is involved in chemo-sensitivity of OVCAs to cisplatin, we analyzed the cell cycle distribution of different OVCAs