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 or/and 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 chemoresistance in OVCAs and offers an alternate regulatory pathway for the development of rationale therapy for cisplatin-resistant ovarian cancer.

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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 cancer 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 gene expression 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.
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). Chk1 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 (Na3VO4) and aprotinin were purchased from Hunter, 2014). Akt2 or pcDNA3 alone as reported previously in Fraser M.

2.2. Cell culture

Cisplatin-sensitive (OV2008 and A2780s) and -resistant (C13* and A2780cp) cell lines and PS3-null SKOV3 cells 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 phospho-chk1 (S345), rabbit total and phosphor-cdc2 (Tyr 15), mouse polyclonal anti-phospho-histone H3 (Serine 10), Rabbit phosphor-cdc2 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). Ribojucie 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.

2.3. 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 Met 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 1:100; 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
treated with or without cisplatin for 24 h. Cisplatin treatment induced an remarkably increase in cell numbers in sub-G1 phase by more than 20% of total cell population in OV2008 and A2780s, which was accompanied by G1 phase arrest. In contrast, resistant OVCAs exposed to cisplatin exerted no increased cells in sub-G1 phase, while significantly accumulated in the S and G2/M phase by 22.79% and 41.48% of total C13* and 26.62% and 33.42% in A2780cp cell population, respectively (Fig. 1B, C). These results confirmed that cisplatin primarily induced apoptosis in OV2008 and A2780s cells. The observation of G2/M phase arrest in C13* and A2780cp cells implies that cisplatin-induced S and G2/M arrest in chemoresistant cells allow more time for reversal of the cytotoxic effects of this drug before entry into mitosis. S or G2/M arrest in response to cisplatin may therefore represent a defense mechanism against the cytotoxic actions of cisplatin. It is well established that the G1 arrest is dependent upon wild-type p53 activity. In C13* (p53 wild type), cisplatin treatment could not induce G1 phase arrest but elicited a cell cycle profile similar to p53 mutant A2780cp cell, suggesting the wild-type p53 in C13* couldn’t be activated, which is consistent with the initiated notion in our lab that accumulated p53 in C13* cells works as “a numbness or dullness”.

3.2. Induction of S and G2/M cycle arrest by cisplatin in chemoresistant OVCAs is associated with S and G2 checkpoint pathway activation

Whereas cisplatin-induced S and G2/M arrest may protect cells from cisplatin-induced cytotoxicity, the linkage between cisplatin exposure and the activation of the S and G2 checkpoint in OVCAs remains unclear. To better define how cisplatin induces S and G2/M arrest, we first analyzed alterations in levels and activation of G2 checkpoint-associated proteins in cisplatin-treated cells. Unsynchronized OVCAs were treated with cisplatin (10 μM) at different times. Immunoblot analyses revealed that there was a transient increase in phosphor-Chk1(S345) level in OV2008 and A2780s cells after cisplatin exposure for 3 to 12 h, then declining rapidly. However cisplatin treatment elicited a later but sustained increase in phosphor-Chk1(S345) level in OV2008 and A2780s cells after cisplatin exposure for 3 to 12 h, then declining slowly. There was no significant change in total Chk1 level in resistant C13* and A2780cp cells but a significant decrease in chemosensitive OV2008 and A2780s cells after 24 h of cisplatin exposure. The similar trends of phosphor-Cdc2(p-Y15) and total Cdc2 levels were observed in these OVCA cells, except that there was no instantaneous induction of phosphor-Cdc2 in OV2008 but inhibition of phosphorylation of Cdc2 at Tyr 15 after 24 h of cisplatin treatment (Fig. 2A, B). Apparently, cisplatin exposure resulted in distinct cellular responses (apoptosis or G2 arrest) in sensitive and resistant OVCAs were correspondent to early/transient or later/sustained induction of the activated and total content of G2 checkpoint-regulatory proteins (Chk1 and Cdc2 kinase) respectively. As sensitive cells used in this experiments with wild-p53 (OV2008, A2780S) and resistance cells contained wild-p53 (C13*) or mutant p53 (A2780cp), it seemed Akt-mediated G2 checkpoint protein and cell cycle regulation were not relevant to p53 status.

Fig. 1. Cisplation treatment resulted in S and G2/M cycle arrest in resistant OVCA cells but remarkable increases in cells in sub-G1 phase in sensitive cells. (A): Chemosensitive and chemoresistant OVCA cells were treated with different concentrations of cisplatin (CDDP) for 24 h, and were collected for Hoechst 33,258 staining to count apoptotic cells by nuclear morphology. (B): OVCA cells were harvested after treatment with 10 μM of cisplatin for 24 h. The cells were fixed and processed for propidium iodide staining to analyze cell cycle progression by flow cytometry, respectively. Representative cell cycle distributions were presented in (B, C). Quantitation of experiment in panel (B). Data were represented as mean ± SEM of four independent determinations. *, p<0.05 relative to CTL group. **, p<0.01 relative to CTL group.
3.3. Dominant negative (DN)-AKT sensitized chemoresistant OVCAs to cisplatin by abrogating S and G2/M arrest, which is associated with inhibition of Chk1 activation

Our previous study showed expression of constitutively active Akt resulted in cancer cells resistance to cisplatin, whereas DN-AKT sensitized the cells to chemotherapeutic drugs in OVCAs (Fraser et al., 2003a, 2003b, 2008). To investigate the potential mechanism under which OVCAs are sensitized to cisplatin, we observed the effect of DN-AKT on chemoresistant C13* cell cycle progression in the absence or presence of the induction of DNA damage. As shown in Fig. 3A, infection with the control LacZ did not affect cell cycle profile, whereas over-expression of DN-AKT induced an obvious increase in the percentage of cells with 4N DNA content in a titer-dependent manner, suggesting that ablation of AKT function through expression of DN-AKT blocked cell cycle at G2/M phase. In contrast, over-expression of DN-AKT abrogated CDDP-induced G2/M arrest while significantly increased the cell proportion in sub-G1 phase, suggesting that these cells were undergoing apoptosis (Fig. 3A). By using the cleavage of the caspase substrate poly (ADP-ribose) polymerase (PARP) as an indicator, we further confirmed that apoptosis was induced, as shown by the cleavage of full-length protein (Fig. 3B). Meanwhile, we observed that about 14% of AKT-depleted cells under cisplatin exposure showed typical nuclear condensation and fragmentation, compared with approximate 1% of control cells (cisplatin treatment alone) (Fig. 3C). These data indicate that AKT-depleted C13* cells arrested at G2/M phase, followed by massive apoptosis. These results could also be observed in p53 mutant A2780cp cells (Fig. 3D, E, F), suggesting DN-AKT induced apoptosis in OVCAs is independent of p53 status. Since Chk1 activation plays essential roles in G2/M arrest, we further detected the phosphor-Chk1 level in DN-AKT infected cells. As is shown in Fig. 3B and E, over-expression of DN-AKT increased phosphorylated Chk1 level, but inhibited cisplatin-induced Chk1 phosphorylation in chemoresistant OVCAs. Cisplatin induced Chk1 activation and resulted in G2 arrest in chemoresistant OVCA cells, which might be a defense mechanism against the cytotoxic actions of cisplatin. We further showed that over-expression of DN-AKT could abrogate cisplatin-induced S and G2/M arrest via inhibiting Chk1 phosphorylation and activation, sensitizing chemoresistant OVCAs to cisplatin.

3.4. Knock-down Chk1 conduced to apoptosis in chemo-resistant cells under cisplatin treatment

To more directly test the association between cisplatin treatment and alterations in G2 checkpoint proteins, RNAi was used to silence Chk1 in resistant C13* cells. We next determined whether Chk1 knock-down influences apoptosis of these cells under cisplatin treatment. As indicated by Western blotting, Chk1 was knocked down efficiently with this approach (Fig. 4A). By using the cleavage of the caspase substrate poly (ADP-ribose) polymerase (PARP) as an indicator, we further confirmed apoptosis was induced, as shown by the cleavage of full-length protein (Fig. 4B). Meanwhile, we observed that about 14% of AKT-depleted cells under cisplatin exposure showed typical nuclear condensation and fragmentation, compared with approximate 1% of control cells (cisplatin treatment alone) (Fig. 3C). These data indicate that AKT-depleted C13* cells arrested at G2/M phase, followed by massive apoptosis. These results could also be observed in p53 mutant A2780cp cells (Fig. 3D, E, F), suggesting DN-AKT induced apoptosis in OVCAs is independent of p53 status. Since Chk1 activation plays essential roles in G2/M arrest, we further detected the phosphor-Chk1 level in DN-AKT infected cells. As is shown in Fig. 3B and E, over-expression of DN-AKT increased phosphorylated Chk1 level, but inhibited cisplatin-induced Chk1 phosphorylation in chemoresistant OVCAs. Cisplatin induced Chk1 activation and resulted in G2 arrest in chemoresistant OVCA cells, which might be a defense mechanism against the cytotoxic actions of cisplatin. We further showed that over-expression of DN-AKT could abrogate cisplatin-induced S and G2/M arrest via inhibiting Chk1 phosphorylation and activation, sensitizing chemoresistant OVCAs to cisplatin.
Fig. 3. DN-AKT induced apoptosis in chemoresistant ovarian cancer cells via inhibiting chk1 activation induced by cisplatin. C13* or A2780cp (p53-mutant chemoresistant) cells were infected with DN-Akt (MOI = 0–80) or LacZ (to equalize total MOI) adenovirus and treated with 10 μM cisplatin (CDDP) or DMSO as control for 24 h. Cells were harvested for following analyses, cell cycle profiles assessed by FACS (A, D), Western blot analysis of PARP cleavage, Chk1 content and phosphorylation (B, E), Hoechst 33,258 staining to count apoptotic cells by nuclear morphology (C, F). Infection of DN-Akt with or without cisplatin treatment induced apoptosis in titre-dependent manner in wild-type and mutant p53 ovarian cancer cells. DN-Akt induced G2/M arrest while abrogated cisplatin-induced S and G2/M arrest. **, p < 0.01 relative to DN-Akt (MOI 0) group.

Fig. 4. Knock-down of Chk1 sensitized tumor cells to cisplation through apoptosis. A, C13* cells and SKOV3 cells were transfected with Chk1 siRNA (100nM), and control siRNA. Forty eight hours after transfection, cells were treated with cisplatin (CDDP) for 24 h, and then harvested for Hoechst staining (B, D) and western blot (A, C). Knock-down chk1 facilitated cisplatin-induced apoptosis of C13* cells (A, B) and SKOV3 cells (C, D).
apoptotic cell counting under microscope according to the nuclear morphology. These results demonstrated that down-regulation of Chk1 mediated cisplatin-induced apoptosis in resistant OVCA cells is independent of p53.

3.5. Constitutively active AKT2 prevented cisplatin-induced apoptosis by delaying G2/M transition in chemosensitive A2780s cells

To further confirm that the role of PI3K/Akt signal in the regulation of cell cycle progression, we used a previously characterized A2780-AAkt2 cell line that constitutively expresses an activated Akt, and a control cell line (A2780-PMH6). As shown in Fig. 5A, treatment of A2780-AAkt2 with cisplatin failed to induce apoptosis while the A2780-PMH6 cells elicited a normal apoptotic response to cisplatin treatment. The cell cycle analysis revealed that cisplatin treatment resulted in significant S and G2/M arrests of Akt-expressing cells while remarkable increases in sub-G1 phase of the control cells (Fig. 5B, C). These results suggest that Akt plays roles in cisplatin-induced S and G2/M checkpoint signaling and in apoptosis inhibition.

Since Chk1 and Cdc2 are critical checkpoint kinases in G2/M arrest, we further investigated if the G2/M arrest in A2780-AAkt2 cells induced by cisplatin is mediated by activating Chk1 and Cdc2. Unsyncronized OVCA were treated with cisplatin (10 μM) at different times. The results in Fig. 5D and E showed that there was a transient increase of phosphor-Chk1(S 345) level in the control A2780-PMH6 cells after cisplatin exposure for 3 to 12 h, then declining rapidly. However cisplatin treatment elicited a sustained increase of phosphor-Chk1 level in resistant A2780-AAkt2 cells, lasting for about 36 h, then declining slowly. There was no significant change in total Chk1 level in resistant A2780-AAkt2 cells but a significant decrease in total Chk1 level in chemosensitive A2780-PMH6 cells after 24 h of cisplatin exposure. The similar trends of phosphor-Cdc2 (p-Y15) protein content were observed in these cells as well. Interestingly, treatment with cisplatin increased total Cdc2 levels in A2780-AAkt2 cells even after 48 h of cisplatin exposure (Fig. 5D, E). These results suggest that cisplatin exposure resulted in distinct cellular responses (apoptosis or G2 arrest) was due to the induction of the activation and content of the known G2 checkpoint-regulatory proteins Chk1 and Cdc2 kinase, which are dependent on the AKT activity status.

4. Discussion

The present study demonstrated that cisplatin exposure elicited S and G2/M cycle arrest in resistant OVCA, in contrast to undergoing apoptosis in sensitive OVCA (Fig. 1). The discrepancy in cellular response to cisplatin was correlated with the different profile of checkpoint kinase activation. Cisplatin elicited a rapid (3 h) activation of Chk1 in sensitive cells, it markedly reduced total and phospho-Chk1 contents over 12 h. However a sustained Chk1 activation was observed in the resistant cells (Fig. 2). As such, this checkpoint pathway may provide the cells a resistance mechanism by limiting further incorporation of the analog and aberrant mitosis, and provide time for repair to occur. Abrogation of the S and G2 phase checkpoint may generate signals that result in cell death, thereby circumventing the cellular defense mechanism and
inducing premature mitosis. So our findings supply a good model to study the potential mechanism of chemoresistance and develop possible new drug target. Apoptosis has been widely used as a measure of chemosensitivity in OVCAs (Gibb et al., 1997). It is well established that PI3-kinase cascade plays role in the regulation of cell growth. Akt, the key downstream effector of the PI3-kinase pathway, is best known for its anti-apoptotic effects. Recently, there are accumulating although controversial evidences showed that PI3K/Akt cascade plays a role in Chk1 activation and G2/M-phase transition in addition to regulation of cell growth and anti-apoptosis. Data from initial reports indicated a function of constitutive active Akt prevents apoptosis and promotes mitotic progression, while inhibition of the PI3-kinase pathway during S phase induced a G2 arrest and apoptosis (Kurosu et al., 2013). In contrast to the initial notion that Akt prevented apoptosis and promoted mitotic progression by inactivate Chk1, a recent report indicates that hematopoietic cytokines enhance Chk1-dependent G2/M checkpoint activation by etoposide through the Akt/GSK3 pathway to inhibit apoptosis (Henry et al., 2001; Jin et al., 2005; Pereira et al., 2015). The apparent contradiction between these reports may be due to the differences in cell types examined, DNA damage presented and the phase in which the cells was hit. Clearly, more studies are required to delineate the exact mechanism by which Akt exerts its effect on the G2/M transition. In the present work, we showed that DN-AKT marked inhibited cisplatin-induced Chk1 activation and G2/M arrest, facilitating apoptosis under cisplatin treatment, while DN-AKT alone increased G2/M arrest, which was associated with slightly activate Chk1. The results suggest that inhibition of Akt exerts opposite effect on G2/M transition, which is dependent on the DNA-damage presented or not.

The resistance of cancer cells to chemotherapy is usually associated with the dysregulated DNA damage repair response. Checkpoint pathways activated in response to DNA damage appear to be central to the response of cells to chemotherapeutic agents and in the ability of tumor cells to develop chemoresistance. DNA damage induces cell cycle delays at the G1/S and G2/M transitions (the G1 and G2 checkpoints, respectively), and a transient decrease in the rate of DNA synthesis (the intra-S checkpoint). The G1 checkpoint is unique in depending primarily on the function of the p53 tumor suppressor protein and its downstream target, the cyclin-dependent kinase inhibitor p21CIP1. G2 arrest, by contrast, is imposed by blocking activation of the mitotic Cdk1-cyclinB complex via inhibition of Cdc25 family phosphatases. Chk1 is a protein kinase that maintains the G2 checkpoint when the cells are under DNA damage conditions. Chk1 kinase is activated on DNA damage by ATR-mediated phosphorylation, which then phosphorylates and inactivates the CDC25 family phosphatases (Smits and Gillespie, 2015; Xiao et al., 2003; Chen et al., 2003). These phosphatases regulate the timely activation of cyclin-dependent kinases at the G1-S and G2-M transitions. In tumor cells that lack p53, the G1 checkpoint is selectively lost, making the cells dependent on its ability to arrest in G2. Abrogation of the G2 checkpoint in the presence of DNA-damaging agents can lead to mitotic catastrophe in the tumor cells (Kawasumi et al., 2014; Kawasaki et al., 2012). Since Chk1 was one of the main mediators of the G2 checkpoint after DNA damage, inhibition of Chk1 provides an attractive opportunity for gene targeted intervention that will abrogate the checkpoint and selectively enhance toxicity of the genotoxic drugs in the cancer cells. Indeed, our observations suggest that inhibition of Akt abrogate cisplatin-induced Chk1 activation, and drive cells into mitosis following DNA-damage induced arrest, resulting in apoptosis (Fig. 3). Knock-down Chk1 enhanced cisplatin-induced apoptosis in C13* and SKOV3 (p53 null) cells (Fig. 4), confirming that Chk1 is essential to chemoresistance and that elimination of Chk1 sensitize OVCAs to cisplatin, which is independent on p53. Furthermore, introduction of constitutive active AKT2 to sensitive OVCAs could reverse its chemosensitivity to cisplatin, and changed the profile of cell cycle and Chk1 activation (Fig. 5). These results strongly suggest that Akt is a culprit in the activation of Chk1, the resultant S and G2/M arrest, and inhibition of apoptosis induced by cisplatin.

An additional point worth noting is that whereas previous studies showed that Chk1 inhibitor-mediated sensitization to various chemotherapeutic agents was selective for cells with nonfunctional p53 (Xu et al., 2011; Blackwood et al., 2013; King et al., 2014). We demonstrated that DN-AKT-mediated sensitization to cisplatin was p53-independent. Our results showed that both p53-wild-type and –mutant resistant OVCAs arrest in S and G2/M phase upon cisplatin treatment by sharing the same profile of cell cycle arrest and checkpoint pathway (Figs. 1-2). DN-AKT induced apoptosis in both cell lines (Fig. 3).

In summary, our work documented a novel role for Akt in the control of G2/M cell cycle progression and showed that inhibition of Akt could overcome the p53-independent G2/M cell cycle checkpoint induced by DNA damage, and promoted apoptosis. This finding on the new activity of Akt in conjuction with its antiapoptotic activity may contribute to genetic stability and could explain its frequent activation in human cancers, especially in resistant cancers. The results also suggest that activation of Akt may influence tumor response to therapy, and that the status of Akt as a prognostic marker should be evaluated in efforts to improve the outcome of ovarian cancer. Chemotherapy combined with inhibitor of Akt could be an efficient strategy for treatment of ovarian cancer.

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