Xeroderma Pigmentosum Group A Promotes Autophagy to Facilitate Cisplatin Resistance in Melanoma Cells through the Activation of PARP1

Rui Ge1,2, Lin Liu1,2, Wei Dai1,2, Weigang Zhang1, Yuqi Yang1, Huina Wang1, Qiong Shi1, Sen Guo1, Xiuli Yi1, Gang Wang1, Tianwen Gao1, Qi Luan1 and Chunying Li1

Xeroderma pigmentosum group A (XPA), a key protein in the nucleotide excision repair pathway, has been shown to promote the resistance of tumor cells to chemotherapeutic drugs by facilitating the DNA repair process. However, the role of XPA in the resistance of melanoma to platinum-based drugs like cisplatin is largely unknown. In this study, we initially found that XPA was expressed at higher levels in cisplatin-resistant melanoma cells than in cisplatin-sensitive ones. Furthermore, the knockdown of XPA not only increased cellular apoptosis but also inhibited cisplatin-induced autophagy, which rendered the melanoma cells more sensitive to cisplatin. Moreover, we discovered that the increased XPA in resistant melanoma cells promoted poly(adenosine diphosphate-ribose) polymerase 1 (PARP1) activation and that the inhibition of PARP1 could attenuate the cisplatin-induced autophagy. Finally, we proved that the inhibition of PARP1 and the autophagy process made resistant melanoma cells more susceptible to cisplatin treatment. Our study shows that XPA can promote cell-protective autophagy in a DNA repair-independent manner by enhancing the activation of PARP1 in melanoma cells resistant to cisplatin and that the XPA-PARP1–mediated autophagy process can be targeted to overcome cisplatin resistance in melanoma chemotherapy.


INTRODUCTION

Melanoma, a malignancy deriving from melanocytes, has the highest mortality rate among skin cancers, with a 5-year survival rate for patients in the advanced stage of only 16% (Eggermont et al., 2014). According to previous studies and clinical observations, chemotherapeutic drugs, including the classic platinum-based drugs like cisplatin, are not very effective in the treatment of melanoma because of the strong drug resistance of melanoma cells (Megahed and Koon, 2014). Thus, clarifying the mechanisms underlying the resistance of melanoma cells to chemotherapeutic drugs such as cisplatin is clinically beneficial to melanoma patients, especially for those in advanced stages.

The antitumor effect of cisplatin has been ascribed to its ability to cause DNA damage, hence inducing cell senescence and apoptosis (Galluzzi et al., 2014). Therefore, adequate and prompt repair of cisplatin-elicted DNA lesions can render tumor cells less susceptible to cisplatin (Bradbury and Middleton, 2004). As a key subunit involved in the nucleotide excision repair (NER) system, xeroderma pigmentosum group A (XPA) protein acts as a central organizer in the NER pathway by recognizing DNA damage and recruiting other NER proteins to DNA lesions (Camenisch et al., 2006; Rademakers et al., 2003). An earlier study on lung adenocarcinoma has reported that XPA can reduce the sensitivity of tumor cells to cisplatin treatment by promoting the repair of DNA lesions and thus preventing cell apoptosis (Wu et al., 2003). However, to our knowledge, the role of XPA in the resistance of melanoma cells to cisplatin treatment has not been investigated.

Autophagy is a cellular process that leads to the degeneration of cytoplasmic components and the recycling of cellular contents and energetic substances (Janku et al., 2011; Kenific and Debnath, 2015). Accumulating evidences suggest that autophagy can protect tumor cells from chemotherapy-caused stress by providing energy and necessary cellular materials (Sui et al., 2013). It is reported that the induction of autophagy in melanoma cells renders the cells less susceptible to the antiproliferative and cytotoxic effects of chemotherapeutic drugs, whereas the suppression of autophagy elevates the sensitivity of melanoma cells to chemotherapy (Del Bollo et al., 2013; Lin et al., 2014). According to previous studies, DNA repair proteins, such as mutL homolog 1 and mutS homolog 2 in the mismatch repair pathway, can influence the survival of tumor cells in chemotherapy not.
only by repairing DNA damage but also through the promotion of the autophagy process (Zeng et al., 2007). Whether XPA, another key DNA repair protein related to the drug resistance of tumor cells, can facilitate resistance to cisplatin by promoting autophagy in tumors like melanoma is still unknown and needs further clarification.

In this study, we initially found that the mRNA level of XPA was elevated in melanoma cells resistant to cisplatin treatment. Subsequent experiments revealed the function of XPA in the resistance of melanoma cells to cisplatin as well as the role of autophagy. We also investigated the downstream mechanism underlying the regulation of XPA on cisplatin-induced autophagy. In addition, we explored the potential therapeutic targets involved in XPA-mediated autophagy for melanoma cells resistant to cisplatin treatment.

**RESULTS**

**XPA contributes to resistance to cisplatin treatment in melanoma cell lines**

To investigate whether XPA takes part in the cisplatin resistance of melanoma cells, we first measured the mRNA expression of XPA and the sensitivity to cisplatin in a panel of six human melanoma cell lines, PIG1 cells (immortalized human epidermal melanocytes), and normal human melanocytes (MC) by quantitative real-time PCR and Cell Counting Kit-8 (Dojingo Molecular Technologies, Rockville, MD) assay, respectively. The mRNA level of XPA in all melanoma cell lines was obviously increased compared with that in the PIG1 cells or MC cells, with the Hs294T, A2058, and WM-266-4 cell lines having the three highest XPA expression levels (Figure 1a). Subsequent cisplatin treatment showed that MC cells were the least sensitive to cisplatin, which was consistent with previous observations that the toxicity of cisplatin on normal tissue is largely limited (Stewart, 2007). Among the malignant cell lines, the half maximal inhibitory concentration of cisplatin in Hs294T and A2058, two cell lines with high mRNA levels of XPA, was much higher than the half maximal inhibitory concentration of cisplatin in other cell lines (Figure 1b and Supplementary Table S1 online), which indicated that XPA may play a role in the resistance of melanoma cells to cisplatin treatment. We then knocked down the expression of XPA in Hs294T and A2058 cells by using small interfering RNAs (siRNAs; knockdown efficiency detected by Western blot test as shown in Figure 1c) and found that the half maximal inhibitory concentration of cisplatin in Hs294T and A2058, two cell lines with high mRNA levels of XPA, decreased 73.6% and 37.7%, respectively (Figure 1c). These results showed that XPA can promote the resistance of melanoma cells to cisplatin treatment.

**XPA prevents cisplatin-treated melanoma cells from undergoing apoptosis**

It has been reported that XPA could prevent cell apoptosis by facilitating the activation of the NER pathway in other malignancies (Wu et al., 2003). Therefore, the phenomenon of XPA contributing to the cisplatin resistance of melanoma cells is probably due to the inhibition of cisplatin-induced cell apoptosis. To test this, the expression of cleaved caspase-3 (p17 fragments), a classical apoptotic marker, was examined in melanoma cell lines by Western blot test. The protein level of cleaved caspase-3 was enhanced in 20 μmol/L cisplatin-treated Hs294T and A2058 cells transfected by XPA siRNA compared with control cells (Figure 2a). Consistently, our apoptosis analysis of
cisplatin-treated Hs294T and A2058 cells determined by flow cytometry showed that the knockdown of XPA led to a higher proportion of early and late apoptotic cells in both cell lines (Figure 2b and 2c). Taken together, these results showed that XPA prevents melanoma cells from cisplatin-induced apoptosis, thus enhancing their resistance to cisplatin treatment. XPA is required for the induction of autophagy in Hs294T and A2058 cells after cisplatin treatment. DNA repair proteins have been proven to promote cell-protective autophagy in tumor cells treated with chemotherapeutic drugs (Zeng et al., 2007), indicating that the cisplatin resistance of melanoma cells mediated by XPA may also result from regulation of the intracellular autophagy process.
Therefore, we examined the autophagy levels of Hs294T and A2058 cells treated with cisplatin. We found that the protein level of light chain protein 3-II (LC3-II), the most widely used biomarker of autophagosome formation, and of XPA in the two strongly resistant cell lines was obviously increased after exposure to 20 μmol/L cisplatin in a time-dependent manner (Figure 3a and 3b). Subsequent ultrastructural transmission electron microscopy analysis showed that cisplatin-treated Hs294T and A2058 cells displayed many cytoplasmic vesicles that had the typical multi-lamellar structure of autophagosomes, whereas fewer autophagic vacuoles were observed in control cells (Figure 3c). These data showed that cisplatin treatment can induce autophagy in resistant melanoma cells.

We went on to investigate whether XPA participated in the autophagy process induced by cisplatin in resistant melanoma cells. Accordant with previous findings, our immunofluorescence assay detected abundant cytoplasmic LC3 puncta in control cells treated with cisplatin. However, after transfection with XPA siRNA, the number of autophagosome-positive cells failed to increase in either the Hs294T or A2058 cell lines after cisplatin treatment (Figure 3d–f). Consistently, our Western blot analysis showed that the protein level of LC3-II in control cells increased along with the extension of cisplatin stimulation time, which, however, could not be observed in XPA-interfered cells (Figure 3g–i). These results verified that XPA is required for the elevated autophagy level induced by cisplatin in resistant melanoma cells.

**XPA mediates cisplatin-induced autophagy through the activation of poly(adenosine diphosphate-ribose) polymerase 1**

Previous studies have reported that poly(adenosine diphosphate-ribose) (PAR) polymerase 1 (PARP1), an early responder to DNA damage, is necessary for the induction of autophagy in cells treated with chemotherapeutic drugs (Munoz-Gamez et al., 2009). Given that the activation of PARP1 could be facilitated by XPA through protein-protein interaction (Fischer et al., 2014; King et al., 2012), PARP1 may be the downstream effector molecule of XPA that is responsible for cisplatin-induced autophagy in resistant melanoma cells. To test this speculation, we detected the content of PAR that is usually used as the marker of PARP1 activation in Hs294T and A2058 cells transfected by XPA siRNA or control siRNA after cisplatin treatment. In cisplatin-treated melanoma cells, the protein level of PAR was obviously up-regulated compared with control cells. However, in XPA-knockdown cells, the expression of PAR was down-regulated, and the addition of cisplatin failed to elevate the expression of PAR, indicating that XPA is indispensable for cisplatin-induced PARP1 activation in resistant melanoma cells (Figure 4a).

To elucidate the role of PARP1 in XPA-mediated autophagy, melanoma cells were treated with PARP1 siRNA or two PARP1 inhibitors, 3,4-dihydro-5-(4-[1-piperidinyl] butoxy)-1(2H)-isoquinolinolone (DPQ) and olaparib, and then the level of LC3-II was measured. As shown in Figure 4b, siRNA against PARP1 significantly inhibited the expression of both PARP1 itself and its activation marker PAR in Hs294T and A2058 cells and restrained the up-regulation of LC3-II after cisplatin treatment. Similarly, the addition of DPQ or olaparib into the culture significantly down-regulated the protein level of PAR and impaired the cisplatin-induced LC3-II elevation in both cell lines (Figure 4c and 4d). The role of PARP1 in autophagy was further substantiated by the observation of fewer cells with LC3 puncta in the cytoplasm after exposure to cisplatin in PARP1-interfered cells compared with control cells (Figure 4e). We went on to detect the expression of genes coding for phosphorylated mechanistic target of rapamycin (mTOR) and high-mobility group protein B1 (HMGB1), which may regulate the autophagy process and had been shown to function downstream of PARP1 (Yang et al., 2015; Zhou et al., 2013) in cisplatin-treated melanoma cell lines. Although the level of phosphorylated mTOR remained the same among all groups (see Supplemental Figure S1a online), the total expression and the cytoplasmic content of HMGB1 were obviously inhibited after the knockdown of PARP1 in cisplatin-treated Hs294T and A2058 cells (see Supplemental Figures S1b and S1c). Taken together, these results proved that XPA promotes autophagy in resistant melanoma cells after cisplatin treatment through facilitating PARP1 activation.

**Inhibition of PARP1-mediated autophagy increases the sensitivity of melanoma cells to cisplatin**

Because autophagy has been proven to provide energy and cellular materials for tumor cells to overcome the stress caused by chemotherapy (Sui et al., 2013), we believed that the autophagy mediated by XPA-PARP1 signaling contributes substantially to the survival of melanoma cells and can be targeted to reduce the cisplatin resistance of cells. Therefore, we used colony formation assay to evaluate the effect of autophagy and PARP1 inhibition on improving the efficacy of cisplatin. The results showed that the number of colonies of both Hs294T and A2058 cells transfected by the siRNA of either autophagy related protein 12, a gene necessary for autophagosome elongation (Tian et al., 2011), or PARP1 (interference efficiency of both siRNAs shown in Figure 5a) was sharply reduced after exposure to cisplatin compared with cells treated with cisplatin alone (Figure 5b and 5c). Moreover, the knockdown of PARP1 seemed to be more efficient than autophagy related protein 12 knockdown in sensitizing melanoma cells to cisplatin-induced long-term cytotoxicity, with almost no colony formation observed in plates treated with both cisplatin and PARP1 siRNA. These data suggested that PARP1-mediated autophagy can be inhibited to eliminate cisplatin resistance in melanoma cells.

**DISCUSSION**

In this study, we found that XPA was highly expressed in melanoma cells strongly resistant to cisplatin and that it prevented melanoma cells from cisplatin-induced apoptosis. Further experiments showed that XPA could promote autophagy through the activation of PARP1. Moreover, we proved that the inhibition of PARP1 as well as XPA-PARP1-mediated autophagy made resistant melanoma cells more susceptible to cisplatin treatment. Our findings indicated that the autophagy mediated by XPA and downstream PARP1 plays a crucial role in the resistance of melanoma cells to cisplatin. Cisplatin and other platinum-based antitumor drugs are classic chemotherapeutic agents applied to a wide range of...
Figure 3. XPA is required for autophagy induced by cisplatin in Hs294T and A2058 cells. (a) Western blots of LC3 and XPA in cells treated with cisplatin for different lengths of time in hours. (b) Band intensity normalized to β-actin is expressed as mean ± standard deviation. (c) Transmission electron microscopy images of autophagic vacuoles in cells treated with cisplatin. The arrows indicate the formed autophagosomes and autolysosomes. Scale bars represent 1 μm (upper row) and 500 nm (lower row). (d–f) Confocal images of LC3 puncta in XPA-knockdown cells with cisplatin treatment. (d) Scale bars represent 5 μm. Statistical analyses are expressed as the mean ± standard deviation; *P < 0.05, **P < 0.01. (g) Western blots of LC3 in XPA-knockdown cells with cisplatin treatment for different lengths of time in hours. The ratio of LC3-II/β-actin is reported as mean ± standard deviation. Ctrl, control; h, hours; LC, light chain; μM, μmol/L; siRNA, single interfering RNA; XPA, xeroderma pigmentosum group A.
Figure 4. XPA promotes cisplatin-induced autophagy through the activation of PARP1. (a) Western blots of LC3, PAR, and XPA in cells transfected with XPA siRNA for 24 hours and then treated with cisplatin. The ratio of LC3-II/β-actin is reported below as mean ± standard deviation. (b–d) Western blots of LC3 and PAR in cells transfected with siRNAs against PARP1 (b) or pretreated with DPQ (c) or olaparib (d) and then treated with cisplatin. The ratio of LC3-II/β-actin is reported below. (e) Confocal images of LC3 puncta in cells transfected with XPA siRNA for 24 hours and then treated with cisplatin. Scale bars represent 5 μm.

Statistical analyses are expressed to the left as mean ± standard deviation; *P < 0.05, **P < 0.01. Ctrl, control; DPQ, 3,4-dihydro-5-[4-(1-piperidinyl) butoxy]-1(2H)-isoquinolinone; LC, light chain; μM, μmol/L; PAR, poly(adenosine diphosphate-ribose); siRNA, single interfering RNA; XPA, xeroderma pigmentosum group A.
tumors. Even though immunotherapy and targeted therapy have been shown to have important antitumor effects in melanoma, platinum-based antitumor drugs are still efficient treatments, especially when they are used in combination with other types of chemotherapy agents or after immunotherapy and targeted therapy (Flaherty et al., 2013). However, the strong resistance of melanoma cells to platinum-based drugs greatly restricts the clinical application of drugs like cisplatin in melanoma. Recent studies have reported that the DNA repair proteins in the NER pathway, such as xeroderma pigmentosum group F and excision repair cross-complementation group 1, contribute to the resistance of melanoma cells to cisplatin (Hatch et al., 2014; McNeil et al., 2015; Song et al., 2011). In addition to xeroderma pigmentosum group F and excision repair cross-complementation group 1, XPA is another NER protein that has been reported to promote the resistance of cancer cells to platinum-based drugs by facilitating a cell-protective NER pathway in

Figure 5. Inhibition of PARP1-mediated autophagy increases the sensitivity of melanoma cells to cisplatin. (a) The efficiency of ATG12 or PARP1 depletion by their respective siRNAs was verified by Western blot test. (b) and (c) The ability of cells transfected with control siRNA, ATG12 siRNA, or PARP1 siRNA to recover after cisplatin withdrawal was assessed with a colony formation assay. Each well shown is a representative image of at least nine similar wells (three independent experiments) (b)). The columns show the number of colonies, and data are expressed as the mean ± standard deviation; *P < 0.05, ***P < 0.001, one-way analysis of variance followed by Newman-Keuls test (c). siRNA, single interfering RNA.
malignancies such as nasopharyngeal carcinoma, lung adenocarcinoma, and prostate cancer (Cummings et al., 2006; Fu et al., 2015; Liu et al., 2012; Wu et al., 2003). In our study, we found that the expression of XPA in Hs294T and A2058 cells, two melanoma cell lines that are highly resistant to cisplatin, was much higher than that in other melanoma cell lines. Furthermore, the inhibition of XPA expression successfully facilitated cisplatin-induced apoptosis in Hs294T and A2058 cells. Our results showed that as a vital part of the NER pathway, XPA prevents melanoma cells from undergoing cisplatin-induced cell death, contributing to the cisplatin resistance of melanoma cells.

As a cellular process that can provide energy and necessary substances for cell survival, autophagy can protect tumor cells during chemotherapy by overcoming the stress caused by chemotherapeutic drugs (Sui et al., 2013). In melanoma, it is reported that the induction of autophagy in chemotherapeutic drug-treated malignant cells renders them less susceptible to the antiproliferative and cytotoxic effects of the drugs, whereas the suppression of autophagy can lead to more cell deaths in melanoma cells exposed to chemotherapeutic drugs (Del Bollo et al., 2013; Lin et al., 2014). Our study proved that autophagy level was upregulated in resistant Hs294T and A2058 melanoma cells treated with cisplatin, which is consistent with previous findings that cisplatin could induce autophagy in cisplatin-resistant tumor cells (He et al., 2015; O’Donovan et al., 2011; Yu et al., 2014). Moreover, we verified that XPA was essential for the induction of autophagy after cisplatin treatment in melanoma cells. Given the protective effect of autophagy on melanoma cells during chemotherapy, our results indicated a potential DNA repair-independent mechanism of XPA in mediating the cisplatin resistance of melanoma cells.

Cisplatin has been reported to induce mitochondrial damage and subsequent depolarization of mitochondrial membrane potential (Mukhopadhyay et al., 2015). Autophagy is a special form of autophagy that can be triggered by dissipated mitochondrial membrane potential-induced activation of the PTEN-induced putative kinase-parkin axis (Ordureau et al., 2014), which indicates that cisplatin treatment can lead to intracellular mitophagy. Nevertheless, several previous studies have detected cisplatin-induced autophagy that is dependent on the up-regulation of the autophagy related protein 14 protein or the activation of the HMGB1-beclin1 pathway, both of which mediate the formation of autophagosomes, specifically in the macroautophagy system (He et al., 2015; Huang et al., 2012). Therefore, the autophagy that we observed in our study should be mainly macroautophagy rather than mitophagy.

The intracellular accumulation of DNA damage can induce the hyperactivation of PARP1 that is an essential factor in DNA repair. Fang et al. (2014) found that PARP1 was more excessively activated in 17-day-old worms with XPA deficiency than in worms of wild type, probably because of adequate accumulation of DNA damage in the XPA-deficient worms. However, they also found that XPA deficiency failed to influence PARP1 activation in 1-day-old worms, which indicated that the hyperactivation of PARP1 must be based on sufficient accumulation of DNA damage, even in cells with XPA deficiency (Fang et al., 2014). This is consistent with our observations that transient knockdown of XPA did not cause PARP1 hyperactivation in melanoma cells. Moreover, we proved that PARP1 could be activated by XPA in cisplatin-treated melanoma cells, possibly because of the binding effect of XPA to the PARP1 protein that has been reported in previous studies (Fischer et al., 2014; King et al., 2012).

As a responder to cytotoxic stresses, activated PARP1 leads to the consumption of oxidized nicotinamide adenine dinucleotide to provide adenosine diphosphate for the formation of PAR subunits (Steffen et al., 2013). It is reported that PARP1 not only promotes DNA repair (especially for the repair of single-strand DNA breaks) in damaged cells but also regulates the process of autophagy (Czarny et al., 2015). Yang et al. (2015) found that the activation of PARP1 facilitates the PARylation of HMGB1 and thus triggers the transportation of HMGB1 into cytoplasm, where HMGB1 promotes autophagy by binding to an autophagy-related protein called beclin 1. However, some other studies have shown that PARP1 regulates autophagy by affecting the activity of the downstream AMP-activated protein kinase-mTOR pathway (Zhou et al., 2013). In our study, we observed that PARP1 knockdown inhibited both the total expression and cytoplasmic translocation of HMGB1, but no obvious change in the phosphorylation level of mTOR was observed. These results support the idea of an HMGB1-dependent but mTOR-independent modulation of PARP1 on autophagy in melanoma cells treated with cisplatin (Figure 6).

Anti-autophagy strategies have been applied in clinical trials on tumors, aiming to overcome the development of resistance to chemotherapies (Sui et al., 2013). Several studies have shown that the inhibition of autophagy enhances cisplatin-induced cell deaths in resistant malignancies (He et al., 2015; Liu et al., 2011; O’Donovan et al., 2011). We also found that the inhibition of autophagy mediated by the XPA-PARP1 pathway sensitized resistant melanoma cells to cisplatin treatment. This phenomenon indicates that olaparib, a PARP1 inhibitor that has already been approved by the U.S. Food and Drug Administration in treating BRCA mutation ovarian cancer (Bixel and Hays, 2015) and can inhibit cisplatin-induced autophagy according to our previous observations, may also be used to prevent or reverse cisplatin resistance in melanoma. More interestingly, we detected a stronger inhibition on the proliferation of cisplatin-resistant melanoma cells in PARP1-knockdown groups compared with that in autophagy related protein 12-knockdown groups. Previous studies have claimed that the inhibitor of PARP1 blocks its function in DNA repair and has antitumor effects, especially on the tumor cells in which the PARP1-mediated DNA repair predominates (Farmer et al., 2005; Sandhu et al., 2010). In addition, PARP1 regulates the activity of some crucial transcription factors, thus influencing the expression and activity of proteins like p53, which is vital in deciding the survival and death of cells (Feng et al., 2015). These findings support the idea that PARP1 can maintain the cell viability of resistant melanoma cells after cisplatin treatment not only by the induction of autophagy but also through other mechanisms such as triggering DNA repair or activating p53. Therefore, PARP1 is a promising target that...
37°C in the presence of 5% CO₂. The second- or third-passage melanocytes were used in all experiments. All foreskin specimens were collected with the approval of the Institutional Review Board of Fourth Military Medical University, Xi’an, China. Written informed consent was obtained from all patients, according to the Declaration of Helsinki. The immortalized normal human epidermal melanocyte cell line PIG1 (a gift from Dr. Caroline Le Poole, Loyola University Chicago, Maywood, IL) was cultured in Medium 254 (Cascade Biologics) supplemented with Human Melanocyte Growth Supplement (Cascade Biologics) and 5% fetal bovine serum (Invitrogen, San Diego, CA) in the presence of 5% CO₂ at 37°C. Human melanoma cell lines WM35, SK-Mel-1, SK-Mel-5, HS294T, A2058, and WM-266-4 were obtained from ATCC (Manassas, VA). Human primary melanoma cells WM35 were cultured in RPMI-1640 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and human metastatic melanoma cell lines SK-Mel-1, SK-Mel-5, HS294T, A2058, WM-266-4 were cultured in Dulbecco’s MEM/F12 medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen). Cisplatin (Sigma-Aldrich, St Louis, MO) was used at a concentration of 20 μmol/L unless specified otherwise. DPQ (Cayman Chemical, Ann Arbor, MI) was used at a concentration of 10 μmol/L. Olaparib (Selleckchem, Houston, TX) was used at a concentration of 10 μmol/L.

**Colony Formation Assay**

The ability of cells to recover from cisplatin treatment and form colonies was evaluated on a monolayer surface. After treatment with 5 μmol/L of cisplatin, adherent cells were trypsinized and counted, and viability was determined. Of those viable cells, 2,000 cells were reseeded in a six-well plate (in triplicate). Cells were allowed to adhere and grow for between 12 and 14 days. To visualize colonies, cells were fixed in 100% ethanol and stained with crystal violet solution (Sigma-Aldrich). Colonies were scored manually.

**CONFLICT OF INTEREST**

The authors state no conflict of interest.

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**SUPPLEMENTARY MATERIAL**

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at http://dx.doi.org/10.1016/j.jid.2016.01.031.

**REFERENCES**


Role of XPA in Cisplatin Resistance of Melanoma


