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The RING heterodimer BRCA1–BARD1 is a ubiquitin ligase inactivated by the platinum-based anticancer drugs

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Abstract The breast cancer susceptibility protein 1 (BRCA1) participates in the maintenance of cells genomic integrity through DNA repair, cell cycle checkpoint, protein ubiquitination, and transcriptional regulation. The N-terminus of BRCA1 contains a RING domain that preferentially forms a heterodimeric complex with BARD1. The BRCA1–BARD1 RING complex has an E3 ubiquitin ligase activity that plays an essential role in response to DNA damage. Preclinical and clinical studies have recently revealed that structural changes to the heterodimer result in alteratinns to the BRCA1-mediated DNA repair pathways in cancer cells, and lead to hypersensitivity to several chemotherapeutic agents. It is of interest to approach the BRCA1 RING domain as a potentially molecular target for platinum-based drugs for cancer therapy. A previous study has shown that the anticancer drug cisplatin formed intramolecular and intermolecular BRCA1 adducts in which His117 was the primary platinum-binding site, and conferred conformational changes and induced thermostability. Here, we have studied the functional consequence of the in vitro platination of the BRCA1 RING domain by a number of platinum complexes. The BRCA1 ubiquitin ligase activity was inhibited by transplatin > cisplatin > oxaliplatin > carboplatin in that order. The consequences of the binding of the platinum complexes on the reactivity of the BRCA1 were also discussed. The data raised the possibility of selectively targeting the BRCA1 DNA repair for cancer therapy.

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Introduction

The breast cancer susceptibility gene 1 (*BRCA1*) is essential for maintaining genomic stability, and is associated with a number of cellular processes, including DNA repair, cell cycle checkpoint, transcriptional regulation, and protein ubiquitination [1, 2]. *BRCA1* encodes for 1,863 amino acid residues with its N-terminus, containing a RING domain that is characterized by a conserved pattern of seven cysteines and one histidine that form two distinct Zn^{2+}-binding sites [3]. The BRCA1 RING domain preferentially forms a heterodimeric complex with another RING domain of BARD1 (the BRCA1-associated RING domain 1) through an extensive four-helix-bundle interface (Fig. 1). The two proteins require each other for their mutual stabilities, and are co-localized in nuclear dots (sites in the nucleus concerned with transcription) during the S-phase of the cell cycle and in nuclear foci (sites associated with repair of DNA caused by damage agents or γ irradiation) [4]. Of further interest is that the BRCA1–BARD1 complex has enzymatic activity of an E3 ubiquitin ligase that specifically transfers ubiquitin (a small peptide) to protein substrates that regulate some aspects of cell biology [5, 6]. Ubiquitination is a form of post-translational modification responsible not only for traditionally targeting proteins for proteasome-dependent degradation but also for playing roles in diverse cellular processes, such as protein transport, and DNA repair [7]. Ubiquitination is a multistep process initiated by an ATP-dependent activation through the formation of a thioester bond between the C-terminal glycine of ubiquitin and a cysteine residue of a

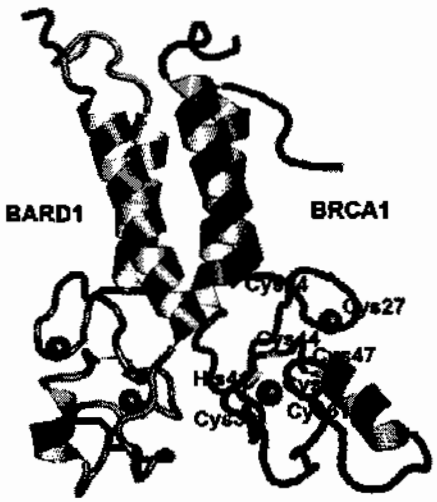

Fig. 1 Solution structure of the BRCA1–BARD1 RING heterodimer. The *spheres* represent Zn^{2+} atoms. The dimerization interface is formed by the antiparallel α-helices, flanking the central RING motif of both BRCA1 and BARD1. The BRCA1 RING domain contains two Zn^{2+}-binding sites in an interleaved fashion, which the first and third pairs of cysteines (Cys24, Cys27, Cys44, and Cys47) form site I, and the second and fourth pairs of cysteines and histidine (Cys39, His41, Cys61, and Cys64) form site II. This structure was generated with PyMOL software (http://pymol.sourceforge.net) based on the protein databank (PDB: 1JM7) [3]

ubiquitin-activating enzyme (E1). The ubiquitin is then transferred to the active cysteine site of a ubiquitin-conjugating enzyme (E2) by transesterification. Finally, ubiquitin is specifically attached to the ε-amino group of a lysine on its protein substrates via an isopeptide bond mediated by a ubiquitin ligase (E3). Candidate substrates for the BRCA1–BARD1 RING complex have recently emerged from in vitro studies, such as BRCA1 itself, nucleosomal histones, RNA polymerase II, and the estrogen receptor α [8–11]. Thus, BRCA1-dependent ubiquitination is probably responsible for modifying many cellular activities. Recently, extensive investigations have examined the relevance of the BRCA1-mediated E3 ubiquitin ligase activity to its tumor suppression function. After a double-strand break (DSB) in DNA, the BRCA1 complex can repair DNA lesions as a result of its co-localization with other DNA repair proteins to the ubiquitinated histone variant γ-H2AX at the sites of DNA damage through the interaction with RAP80 and phosphorylated Abraxas [12]. Many cancer-predisposing mutations in the BRCA1 RING domain, that inhibited the E3 ligase activity and its ability to accumulate at damaged sites, were defective in the homologous recombination that is critical for tumor suppression [13, 14]. Interestingly, BRCA1 accumulation at the sites of DSB occurred rapidly (within 20 s), and it required the RING structure (residue 1–200 of BRCA1) for the rapid recruitment, with Ku80, at damaged sites in response to non-homologous end joining [15]. Missense mutations in the BRCA1 RING domain significantly reduced their accumulations at the DSB, and abolished the association with Ku80. Therefore, the loss of the BRCA1 E3 ligase activity rendered cancerous cells hypersensitive to ionizing radiations to indicate a significant role for ubiquitination in the DNA damage response [16].

Recently, a new approach for cancer therapy involves alterations to the DNA repair pathways in which the cancer cells with dysfunctional DNA repair pathways accumulate high levels of DNA damage that eventually result in major genomic instability and cell death [17]. Several preclinical and clinical studies have demonstrated the utilization of a dysfunctional BRCA1 as a clinically validated target for breast and ovarian cancer treatment [18–20]. The E3 ubiquitin ligases that play essential roles in the regulation of many cellular processes, including DNA repair, have now become potential cancer drug targets and prognostic biomarkers [21, 22]. Recently, it has been reported that the anticancer drug cisplatin affected the conformation of the apo-form of the BRCA1 RING finger domain [23]. Mass spectrometric (MS) analyses revealed that cisplatin formed intramolecular and intermolecular BRCA1 adducts, and that a preferential platinum-binding sites was located on histidine 117 [23]. An enhanced thermostability of BRCA1 was also observed after protein platination. To raise the possibility that the BRCA1 RING domain could be a molecular target for platinum-based drugs, we further investigated the functional consequences of the platinated BRCA1 on its effect on the E3 ubiquitin ligase activity.

Materials and methods

Plasmid construction and protein purification

The short N-terminal fragment of the BRCA1 protein amino acid residues 1–304, and those of the BARD1 protein amino acid residues 26–327 (Addgene plasmid 12646) were produced as a GST fusion by cloning the respective genes into the bacterial plasmid pGEX-4T1 (Amersham Biosciences). The full-length *ubiquitin* (*Ub*) (Addgene plasmid 12647) and *UbcH5c* (Addgene plasmid 12643) genes were inserted into a pET28a(+) derivative for expression of a His_6-tagged protein. All recombinant plasmids were verified by DNA sequencing, and transformed into *Escherichia coli* BL21(DE3) for production of the protein. Proteins expression was induced with 0.5 mM isopropyl-1-thio-β-D-galactopyranoside for 12 h at 25°C. Cell pellets were resuspended in a lysis buffer [50 mM Tris (pH 7.4), 50 mM NaCl, 10% glycerol, 10 mM DTT, 1% Triton X-100, 0.5% NP-40, and 1 mM PMSF], and then lysed by sonication.

GST-tagged proteins were freshly prepared by binding to a glutathione-agarose column (Amersham Biosciences). The bound proteins were eluted with a buffer, containing 50 mM Tris (pH 7.4), 10 mM β-mercaptoethanol, and 20 mM reduced glutathione, and the purified proteins were extensively dialyzed against deionized water. His_6-tagged proteins were purified using nickel beads (Qiagen), and the bound proteins were first washed with a binding buffer [50 mM Tris (pH 7.4), 50 mM NaCl, and 10 mM imidazole] before being eluted with the binding buffer containing 300 mM imidazole. Purified His_6–Ub and His_6–UbcH5c proteins were then dialyzed against a buffer, containing 50 mM Tris (pH 7.0), 10 mM β-mercaptoethanol, and 10% glycerol. Human His_6-E1 enzyme was purchased from Enzo Life Sciences.

Preparation of the platinum–BRCA1 complexes

Cisplatin, transplatin, carboplatin, and oxaliplatin were purchased from Sigma-Aldrich, and prepared as stock solutions in deionized water (Fig. 2). Purified BRCA1 proteins (1.67 μM) were mixed with the platinum compounds at concentration of 5–1000 μM. The reaction mixtures were incubated in the dark for 24 h at 4°C to help to maintain BRCA1 activity and stability. Samples were subjected to extensive ultrafiltration using Macrosep centrifugal devices (Pall Life Sciences) to remove any unbound platinum. The amount of protein was then carefully determined by the Bradford assay, using BSA as standard.

In vitro ubiquitin ligase assay

The ubiquitin ligase reactions (20 μl) contained 20 μM Ub, 300 nM E1, 5 μM UbcH5c, 2 μg BRCA1 or BRCA1 adducts, and 2 μg BARD1 in a buffer [50 mM Tris (pH 7.5), 0.5 mM DTT, 5 mM ATP, 2.5 mM $MgCl_2$, and 5 μM $ZnCl_2$]. Two separate reactions were incubated at 37°C for 3 h, and then terminated by adding an equal volume of SDS-loading dye before electrophoresis on 8% SDS-PAGE and visualization of the protein bands using silver-staining. The relative E3 ligase activity of BRCA1 adducts was quantified by normalizing the density of an apparent band of the ubiquitinated-protein conjugates to that of the parental BRCA1 as the control, using a Bio-Rad GS-700 Imaging Densitometer.

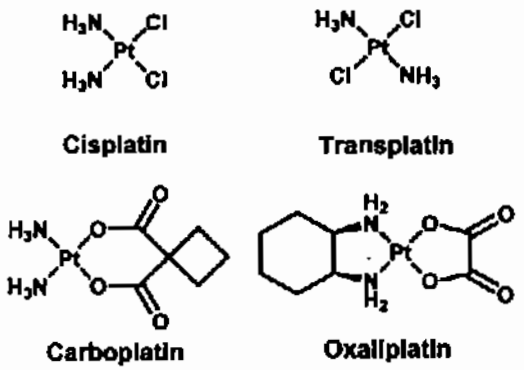

Fig. 2 Molecular structures of the platinum complexes used in this study

Results and discussion

BRCA1 exhibited the E3 ubiquitin ligase activity

The BRCA1–BARD1 RING complexes in the presence of ATP, ubiquitin, E1, and UbcH5c exhibited E3 ubiquitin ligase enzyme activity that promoted the formation of high molecular weight polyubiquitin species that was significantly greater than those produced by the individual BRCA1 or BARD1 RING domain (Fig. 3). The apparent ubiquitinated products, migrating at ~70, 80, and 212 kDa, were likely to be the free ubiquitin polymer or autoubiquitinated BRCA1 or BARD1 conjugates, respectively [9]. Previous

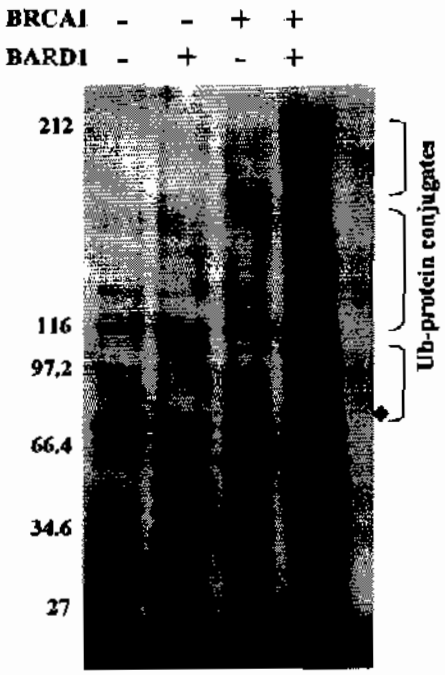

Fig. 3 In vitro ubiquitination. Complete reaction mixtures, containing 20 μM Ub, 300 nM E1, 5 μM UbcH5c, 2 μg BRCA1 (residues 1-304), and 2 μg BARD1 (residues 26-327), were incubated at 37°C for 3 h. Lack of BRCA1 and/or BARD1 components in the reactions were carried out under the same conditions. Samples were then resolved on 8% silver-stained SDS-PAGE. Molecular weight markers are also identified. An apparent ubiquitinated product was indicated by *filled diamond*

studies have demonstrated that BRCA1 and BARD1 formed a stable heterodimeric complex through an interaction between their RING domains [3]. The complex provided the proper contact surface on the BRCA1 in the first and second Zn^{2+}-loops, and in the central helix of the RING for binding E2/UbcH5c. The substantial E3 ligase activity of the BRCA1 complexes may result from the proper positioning and stabilization of the BRCA1 RING domain relative to the binding interfaces of both the BARD1 and UbcH5c [24].

BRCA1-mediated E3 ligase inactivated by the platinum-based drugs

To gain further insights into the functional consequence of the platinated BRCA1, the BRCA1 RING protein was platinated in vitro by cisplatin, transplatin, carboplatin, and oxaliplatin at various concentrations. The results showed that the relative E3 ligase activity was inversely proportional to the concentration of the platinum complexes (Fig. 4). An increase in platinum concentration was accompanied by a high amount of BRCA1 adducts and a low amount of native BRCA1 protein as described previously [23]. To address whether the inhibition of the E3 ligase activity resulted from the formation of BRCA1 adducts or a reduced amount of the BRCA1 subunit, a ten-fold excess amount of the platinated BRCA1 was assayed for the E3 ligase activity. The result demonstrated that the platination of BRCA1 was indeed involved in the inhibition of the E3 ligase activity (lane 3, Fig. 5). In a similar way, the platinum-BARD1 adducts failed to exhibit any E3 ligase activity (lane 4 and 5, Fig. 5).

Transplatin, a clinically ineffective *trans*-platinum complex, was the most promising agent to completely abolish the E3 ligase activity at its effective concentration of 80 μM compared to cisplatin and oxaliplatin (100 and 250 μM, respectively) (Fig. 4). Under the same experimental condition, partial E3 ligase activity was still observed for the carboplatin-BRCA1 adducts at concentrations that exceeded 1000 μM. The E3 ligase activity was reduced by half at concentrarions of 53 μM for transplatin, 60 μM for cisplatin, 150 μM for oxaliplatin, and 780 μM for carboplatin, respectively (Fig. 6). As a result, the reactivity of the platinum complexes towards the BRCA1 RING domain decreased in the following order: transplatin > cisplatin > oxaliplatin > carboplatin. The geometry and the properties of both the leaving and the non-leaving groups of the platinum complexes seemed ro play essential roles in controlling the reactivity towards BRCA1. The activation of the platinum complexes occurs when the leaving group either chloride or oxygen is replaced by water before the interaction with the nucleophilic groups of

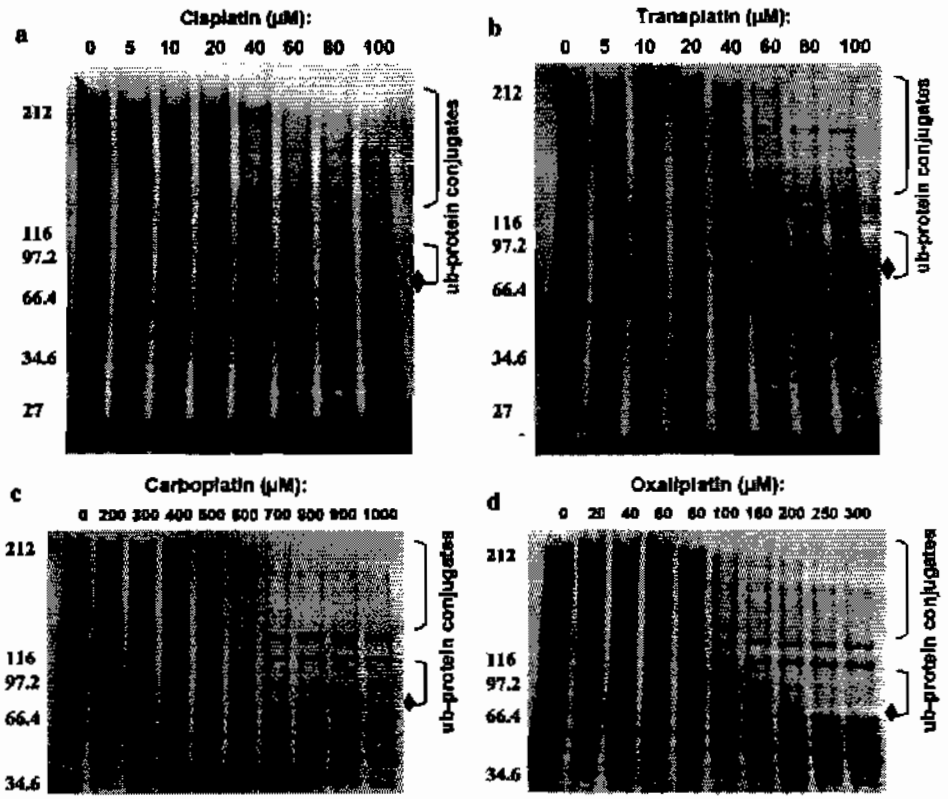

Fig. 4 In vitro ubiquitin ligase activity of the platinum–BRCA1 complexes. Two μg of the platinum-BRCA1 adducts with a number of defined concentrations of cisplatin (**a**), transplatin (**b**), carboplatin (**c**), and oxaliplatin (**d**) was assayed for the ubiquitin ligase activitiy. An apparent ubiquitinated product (as indicated by *filled diamond*) was markedly reduced as the concentration of platinum increased

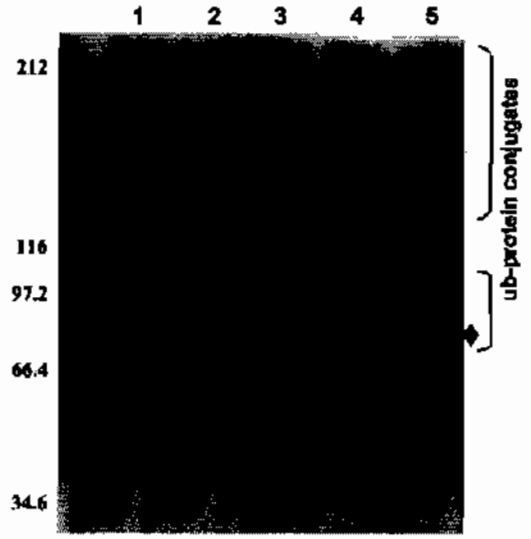

Fig. 5 In vitro ubiquitin ligase activity of the platinum-BRCA1 or platinum-BARD1 complexes. *Lane 1* complete E3 ligase reaction with 2 μg native BRCA1, *lane 2* reaction of 2 μg BRCA1 adduct with 100 μM cisplatin, *lane 3* reaction of 20 μg BRCA1 adduct with 100 μM cisplatin, *lane 4* reaction of 2 μg BARD1 adduct with 100 μM cisplatin, and *lane 5* reaction of 20 μg BARD1 adduct with 100 μM cisplatin. An apparent ubiquitinated product (as indicated by *filled diamond*) was markedly reduced as the concentration of cisplatin increased (Fig 4a)

the protein side-chains [25]. The chelation effect and steric hindrance by the bulky leaving ligand of cyclohutane-1,2-dicarboxylic acid results in a much slower hydrolysis rate (5.00×10^{-9} s^{-1} for carboplatin *vs* 1.62×10^{-5} s^{-1} for cisplatin), and indicates that there is a very low reactivity of carboplatin to BRCA1 [26, 27]. The chloro leaving groups in both cisplatin and transplatin readily undergo hydrolysis in water, and this results in a higher reactivity. The replacement of two chlorides in the *trans* configuration of transplatin is the easiest process compared to that of cisplatin. Transplatin is, therefore, the most effective compound in inhibiting the E3 ligase activity. We suggest that the *cis/trans* geometry plays an important role in the reactivity and interaction with proteins [28]. In addition, oxaliplatin exhibits a moderate reactivity to BRCA1 because the hydrolysis rate of the oxalate exchangeable group (1.20×10^{-6} s^{-1}) is intermediate between those reported for cisplatin and carboplatin under similar conditions [29]. The five-membered chelation and the steric hindrance of the diamminecyclohexane non-leaving moiety at the platinum center of oxaliplatin are believed to be unhydrolyzable, and result in its significant stability [29]. This result was consistent with previous studies that have demonstrated a similar reactivity of the platinum complexes to a number of oligonucleotides and proteins, such as ubiquitin, human serum albumin, and a human copper transporter 1 [25, 28, 30, 31].

Preclinical and clinical studies have recently gained much attention by taking advantage of the inherent weakness of the BRCA1 dysfunction in cancer cells that increases their sensitivity to DNA-damaging agents, such as platinum agents. A pathology determined complete response and excellent compliance was observed in nine (90%) out of 10 breast cancer patients with *BRCA1* mutations after cisplatin chemotherapy every 3 weeks for four cycles [19]. Irrespective of the *BRCA1* status, eighteen (64%) of 28 triple-negative breast cancer patients also had a good clinical response to neoadjuvant therapy with cisplatin [32]. Factors associated with a good cisplatin response included young age, low *BRCA1* mRNA expression, *BRCA1* promoter methylation, p53 mutations, and a gene expression signature of the activity of E2F3. In addition, the significant benefits of the pathological response and overall survival rate from cisplatin-based chemotherapy were extended to the bladder, ovarian, and non-small cell lung (NSCL) cancers patients [33–35]. Therefore, platinum-based therapy appears to be effective in a high proportion of patients with a BRCA1 dysfunction.

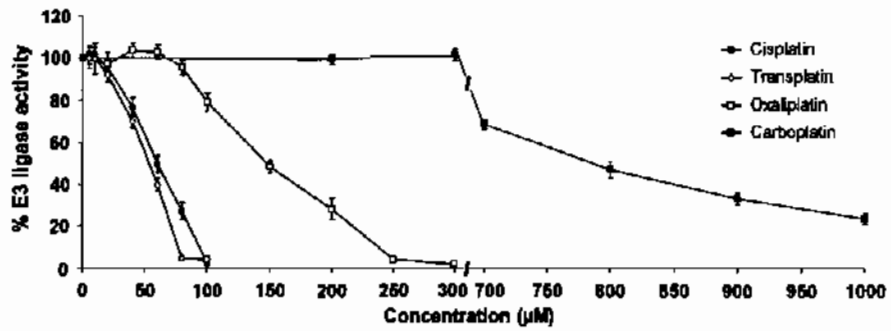

Fig. 6 Inhibition of the BRCA1 E3 ligase activity by the platinum complexes. The apparent ubiquitinated products (as indicated by *filled diamond*) in gels shown in Fig. 4 were quantified by Bio-Rad GS-700 Imaging Densitometer. The relative E3 ligase activity of BRCA1 adducts (%) was plotted as a function of the concentration of the platinum complexes

This study has revealed the inactivation of a BRCA1 function by platinum complexes. This could raise the possibility of utilizing the BRCA1 RING domain as a potentially molecular target for platinum-based agents in cancer chemotherapy. A new generation of platinum compounds, especially *trans*-platinum ones, or other metal-based drugs that specifically target the BRCA1 RING domain, and disrupt its E3 ligase activity could significantly improve the efficacy of the anticancer drugs [36].

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References

1. Huen MSY, Shirley MH, Chen J (2010) BRCA1 and its toolbox for the maintenance of genome integrity. Nat Rev Mol Cell Biol 11:138–148. doi:10.1038/nrm2831
2. O'Donovan PJ, Livingston DM (2010) BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis 31:961–967. doi:10.1093/carcin/bgq069
3. Brzovic PS, Rajagopal P, Hoyt DW, King MC, Klevit RE (2001) Structure of a BRCA1-BARD1 heterodimeric RING-RING complex. Nat Struct Biol 8:833–837. doi:10.1038/nsb1001-833
4. Wu LC, Wang ZW, Tsan JT, Spillman MA, Phung A, Xu XL, Yang MCW, Hwang LY, Bowcock AM, Baer R (1996) Identification of a RING protein that can interact in vivo with the *BRCA1* gene product. Nat Genet 14:430–440. doi:10.1038/ng1296-430
5. Hashizume R, Fukuda M, Maeda I, Nishikawa H, Oyake D, Yabuki Y, Ogata H, Ohta T (2001) The RING heterodimer BRCA1-BARD1 is a ubiquitin ligase inactivated by a breast cancer-derived mutation. J Biol Chem 276:14537–14540. doi:10.1074/jbc.C000881200
6. Xia Y, Pao GM, Chen H-W, Verma IM, Hunter T (2003) Enhancement of BRCA1 E3 ubiquitin ligase activity through direct interaction with the BARD1 protein. J Biol Chem 278:5255–5263. doi:10.1074/jbc.M204591200
7. Bergink S, Jentsch S (2009) Principles of ubiquitin and SUMO modifications in DNA repair. Nature 458:461–467. doi:10.1038/nature07963
8. Wu-Baer F, Ludwig T, Baer R (2010) The UBXN1 protein associates with autoubiquitinated forms of the BRCA1 tumor suppressor and inhibits its enzymatic function. Mol Cell Biol 30:2787–2798. doi:10.1128/MCB.01056-09
9. Mallery DL, Vandenberg CJ, Hiom K (2002) Activation of the E3 ligase function of the BRCA1/BARD1 complex by polyubiquitin chains. EMBO J 21:6755–6762. doi:10.1093/emboj/cdf691
10. Starita LM, Horwitz AA, Keogh MC, Ishioka C, Parvin JD, Chiba N (2005) BRCA1/BARD1 ubiquitinate phosphorylated RNA polymerase II. J Biol Chem 280:24498–24505. doi:10.1074/jbc.M414020200
11. Eakin CM, MacCoss MJ, Finney GL, Klevit RE (2007) Estrogen receptor α is a putative substrate of the BRCA1 ubiquitin ligase. Proc Natl Acad Sci USA 104:5794–5799. doi:10.1073/pnas.0610887104
12. Morris JR (2010) More modifiers move on DNA damage. Cancer Res 70:3861–3862. doi:10.1158/0008-5472.CAN-10-0468
13. Morris JR, Boutell C, Keppler M, Densham R, Weekes D, Alamshah A, Butler L, Galanty Y, Pangon L, Kiuchi T, Ng T, Solomon E (2009) The SUMO modification pathway is involved in the BRCA1 response to genotoxic stress. Nature 462:886–890. doi:10.1038/nature08593
14. Ransburgh DJ, Chiba N, Ishioka C, Toland AE, Parvin JD (2010) Identification of breast tumor mutations in BRCA1 that abolish its function in homologous DNA recombination. Cancer Res 70:988–995. doi:10.1158/0008-5472.CAN-09-2850
15. Wei L, Lan L, Hong Z, Yasui A, Ishioka C, Chiba N (2008) Rapid recruitment of BRCA1 to DNA double-strand breaks is dependent on its association with Ku80. Mol Cell Biol 28:7380–7393. doi:10.1128/MCB.01075-08
16. Ruffner H, Joazeiro CAP, Hemmati D, Hunter T, Verma IM (2001) Cancer-predisposing mutations within the RING domain of BRCA1: loss of ubiquitin protein ligase activity and protection from radiation hypersensitivity. Proc Natl Acad Sci USA 98:5134–5139. doi:10.1073/pnas.081068398
17. Amir E, Serugn B, Serrano R, Ocanu A (2010) Targeting DNA repair in breast cancer: a clinical and translational update. Cancer Treat Rev. doi:10.1016/j.ctrv.2010.03.006
18. Tassone P, Martino MTD, Ventura M, Pietragalla A, Cucinotto I, Calimeri T, Bulotta A, Neri P, Caraglia M, Tagliaferri P (2009) Loss of BRCA1 function increases the antitumor activity of cisplatin against human breast cancer xenografts in vivo. Cancer Biol Ther 8:648–653. doi:10.4161/cbt.8.7.7968
19. Byrski T, Huzarski T, Dent R, Gronwald J, Zuziak D, Cybulski C, Kladny J, Gorski B, Lubinski J, Narod SA (2009) Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat 115:359–363. doi:10.1007/s10549-008-0128-9
20. Price M, Monteiro ANA (2010) Fine tuning chemotherapy to match BRCA1 status. Biochem Pharmacol 80:647–653. doi:10.1016/j.bcp.2010.05.015
21. Sun Y (2006) E3 ubiquitin ligases as cancer targets and biomarkers. Neoplasia 8:645–654. doi:10.1593/neo.06376
22. Hoeller D, Dikic I (2009) Targeting the ubiquitin system in cancer therapy. Nature 458:438–444. doi:10.1038/nature07960
23. Atipairin A, Canyuk C, Ratanaphan A (2010) Cisplatin affects the conformation of apo-form, not holo-form, of BRCA1 RING finger domain and confers thermal stability. Chem Biodivers 7:1949–1967. doi:10.1002/cbdv.200900308
24. Brzovic PS, Keeffe JR, Nishikawa H, Miyamoto K, Fox D III, Fukuda M, Ohta T, Klevit R (2003) Binding and recognition in the assembly of an active BRCA1/BARD1 ubiquitin-ligase complex. Proc Natl Acad Sci USA 100:5646–5651. doi:10.1073/pnas.0836054100
25. Wu Z, Lin Q, Liang X, Yang X, Wang N, Wang X, Sun H, Lu Y, Guo Z (2009) Reactivity of platinum-based antitumor drugs towards a Met- and His-rich 20 mer peptide corresponding to the N-terminal domain of human copper transporter 1. J Biol Inorg Chem 14:1313–1323. doi:10.1007/s00775-009-0576-7
26. Frey U, Ranford JD, Sadler PJ (1993) Ring-opening reactions of the anticancer drug carboplatin: NMR characterization of cis-[Pt(NH_3)$_2$(CBDCA-O)(5′-GMP-N7)] in solution. Inorg Chem 32:1333–1340. doi:10.1021/ic00060a005
27. Davies MS, Berners-Price SJ, Hambley TW (2000) Slowing of cisplatin aquation in the presence of DNA but not in the presence of phosphate: improved understanding of sequence selectivity and the roles of monoaquated and diaquated species in the binding of cisplatin to DNA. Inorg Chem 39:5603–5613. doi:10.1021/ic000847w
28. Trynda-Lemiesz L, Kozłowski H, Keppler BK (1999) Effect of *cis*-, *trans* diamminedichloroplatinum(II) and DBP on human

serum albumin. J Inorg Biochem 77:141–146. doi:10.1016/S0162-0134(99)00183-X
29. Jerremalm E, Videhult P, Alvelius G, Griffiths WJ, Bergman T, Eksborg S, Ehrsson H (2002) Alkaline hydrolysis of oxaliplatin-isolation and identification of the oxalato monodentate intermediate. J Pharm Sci 91:2116–2121. doi:10.1002/jps.10201
30. Casini A, Gabbiani C, Michelucci E, Pieraccini G, Moneti G, Dyson PJ, Messori L (2009) Exploring metallodrug-protein interactions by mass spectrometry: comparisons between platinum coordination complexes and an organometallic ruthenium compound. J Biol Inorg Chem 14:761–770. doi:10.1007/s00775-009-0489-5
31. Groessl M, Tsybin YO, Hartinger CG, Keppler BK, Dyson PJ (2010) Ruthenium versus platinum: interactions of anticancer metallodrugs with duplex oligonucleotides characterized by electrospray ionisation mass spectrometry. J Biol Inorg Chem 15:677–688. doi:10.1007/s00775-010-0635-0
32. Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li O, Juul N, Leong C-O, Calogrias D, Buraimoh A, Fatima A, Gelman RS, Ryan PD, Tung NM, Nicolo AD, Ganesan S, Miron A, Colin C, Sgroi DC, Ellisen LW, Winer EP, Garber JE (2010) Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 28:1145–1153. doi:10.1200/JCO.2009.22.4725
33. Font A, Taron M, Gago JL, Costa C, Sánchez JJ, Carrato C, Mora M, Celiz P, Perez L, Rodríguez D, Gimenez-Capitan A, Quiroga V, Benlloch S, Ibarz L, Rosell R (2010) BRCA1 mRNA expression and outcome to neoadjuvant cisplatin-based chemotherapy in bladder cancer. Ann Oncol. doi:10.1093/annonc/mdq333
34. Quinn JE, James CR, Stewart GE, Mulligan JM, Wilde P, Chang GK, Mullan PB, Johnston PG, Wilson RH, Harkin DP (2007) BRCA1 mRNA expression levels predict for overall survival in ovarian cancer after chemotherapy. Clin Cancer Res 13:7413–7420. doi:10.1158/1078-0432.CCR-07-1083
35. Taron M, Rosell R, Felip E, Mendez P, Souglakos J, Ronco MS, Queralt C, Majo J, Sanchez JM, Sanchez JJ, Maestre J (2004) BRCA1 mRNA expression as an indicator of chemoresistance in lung cancer. Hum Mol Genet 13:2443–2449. doi:10.1093/hmg/ddh260
36. Aris SM, Farrell NP (2009) Towards antitumor active transplatinum compounds. Eur J Inorg Chem 2009:1293–1302. doi:10.1002/ejic.200801118