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XRCC1 Arg399Gln and Arg194Trp polymorphisms regulate XRCC1 expression and chemoresistance of non-small cell lung cancer cells

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Abstract: X-ray repair cross-complementing protein 1 (XRCC1) could repair cisplatin-induced DNA damage. XRCC1 Arg399Gln and Arg194Trp variants alter XRCC1 expression and function, leading to changes in cancer sensitivity to cisplatin treatment. This study aimed to investigate the effects of XRCC1 Arg399Gln and Arg194Trp polymorphisms on cell viability, apoptosis and XRCC1 expression in cisplatin-sensitive A549 and cisplatin-resistant A549/DDP non-small cell lung cancer (NSCLC) cells. Plasmids carrying XRCC1 Arg399Gln and Arg194Trp were constructed and transfected into A549 and A549/DDP cells. RT–PCR, Western blot, MTT assay, and flow cytometry analysis were performed to assess cell viability, apoptosis, and XRCC1 expression. Compared to control cells, the viability of A549 and A549/DDP cells transfected with XRCC1 Arg399Gln and Arg194Trp was higher and the apoptosis rate was lower, and XRCC1 mRNA and protein expression levels were significantly higher. In conclusion, our results suggest that XRCC1 Arg399Gln and Arg194Trp polymorphisms change XRCC1 expression in NSCLC cells and alter the sensitivity of NSCLC to cisplatin-based chemotherapy.

Introduction

Lung cancer is a significant cause of cancer-related death globally. Histologically, lung cancer can be mainly classified into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Despite recent advances in targeted and immunotherapy of lung cancer, platinum-based chemotherapy is still the primary treatment selection for advanced and metastatic NSCLC (Wen *et al.*, 2016). The cytotoxic effect of platinum is mediated by the formation of bulky intra-strand platinum–DNA adducts, and DNA repair enzymes play an essential role in the repair of DNA adducts (Galluzzi *et al.*, 2012). Therefore, further investigation of expression, function, and polymorphisms of DNA repair genes could predict the efficiency of platinum-based chemotherapy.

X-ray repair cross-complementing gene 1 (XRCC1) is a critical protein in base-excision repair (BER) and single-strand break repair (SSBR) pathways and regulates the sensitivity of tumor cells to irradiation (Hanssen-Bauer *et al.*, 2012). XRCC1 does not possess any enzymatic activity but functions as a scaffold to coordinate with other DNA repair enzymes in the BER/SSBR, including auto-modified poly (ADP-ribose) polymerase 1 (PARP-1) and DNA glycosylase III (Moser *et al.*, 2007).

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Single nucleotide polymorphisms (SNPs) of DNA repair genes may modulate DNA repair capacity and contribute to various diseases (Nemer *et al.*, 2018). To date, XRCC1 variants have been extensively studied for possible association with cancer risk and drug resistance (Li *et al.*, 2012). There are three important SNPs in the coding region of human *XRCC1*: Arg194Trp (R194W or rs1799782), Arg280His (R280H or rs25489), and Arg399Gln (R399Q, rs25487). Among them, XRCC1 Arg399Gln polymorphism has been extensively studied. XRCC1 Arg399Gln polymorphism leads to the substitution of the residue at 399 from arginine to glutamine, impacting DNA-repair complex assembly or capacity (Abdel-Rahman and El-Zein, 2000). Our previous study demonstrated that NSCLC patients carrying XRCC1 399Arg/Arg genotype responded better to platinum-based therapy and could predict a favorable treatment outcome (Li *et al.*, 2012). However, meta-analyses have failed to show any associations between XRCC1 polymorphisms and response to treatment, especially for XRCC1 Arg194Trp (Yuan *et al.*, 2010). This might be partially due to population differences in both frequency and distribution of SNPs. Therefore, in this study we aimed to investigate the impacts of XRCC1 Arg399Gln and Arg194Trp on NSCLC cell viability, apoptosis, and XRCC1 expression. We determined the effects of XRCC1 Arg399Gln and Arg194Trp SNPs in cisplatin-sensitive NSCLC A549 and cisplatin-resistant NSCLC A549/DDP cells.

Materials and Methods

Cell lines and culture

Human cisplatin-sensitive NSCLC A549 and cisplatin-resistant A549/DDP cell lines were obtained from Laboratory of Transplantation Immunity, Sichuan University (Chengdu, China) and cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), L-glutamine (2 mM), sodium pyruvate (1 mM), and penicillin (100 U/mL) at 37°C in a humid incubator with 5% CO_2 as described previously (Wang and He, 2018). For A549/DDP cells, 2 mg/mL cisplatin (Jin Yao Pharmaceutical Company, Tianjin, China) was added to the medium.

Plasmids and transfection

Plasmids were constructed by Ying Run Biological Technology Co. Ltd. (Changsha, China). Two mutants (XRCC1 R194W at codon 194 and R399Q at codon 399) were constructed by site-directed mutagenesis and confirmed by DNA sequencing (plasmid carrying XRCC1 R194W mutation was named pDoubleEx-EGFP-XRCC1 R194W, and plasmid carrying R399Q mutation was named pDoubleEx-EGFP-XRCC1 R399Q). The empty plasmid carrying green fluorescent protein (GFP) was used as a negative control. All plasmids were transfected into A549 and A549/DDP cell lines using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Cell viability MTT assay

Cell viability was examined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously (Tian *et al*., 2017). Briefly, cells were treated with different concentrations of cisplatin (2, 4, or 6 μg/mL) for up to 48 h. Then 20 μL MTT (5 mg/mL) was added and incubated for 4 h and then replaced with 150 μL dimethyl sulfoxide (DMSO). Next, the optical density (OD) at 490 nm was measured by using an ELISA reader (Thermo Fisher Scientific, Austria). The experiments were in triplicate and repeated three times. The cell inhibition rate was calculated as [(OD control-OD experimental)/OD control] × 100%.

Flow cytometric apoptosis assay

Cell apoptosis was examined by using Annexin V-APC/7-AAD apoptosis kit (LianKe Biological Technology Co. Ltd). Briefly, cells were seeded into 24-well plates at 0.5×10^5 cells/well and treated with 2 μg/mL cisplatin for 48 h. Cells were then washed twice with phosphate buffered saline (PBS) and single-cell suspensions were fixed with ice-cold 70% alcohol at 4°C overnight. The cells were washed twice with PBS and incubated with 500 μL of the staining solution at 37°C for 30 min. Cells were then washed with PBS and collected in 500 μL PBS for measurement on a FACS420 flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA). Each assay was performed in triplicate and repeated at least three times.

Reverse transcription-PCR

Total RNA was isolated by using Trizol reagent (Invitrogen). Approximately 1 μg RNA sample was reversely transcribed into cDNA using a RevertAid first strand cDNA synthesis kit (Thermo, Waltham, MA, USA). PCR was performed using DyNAMO Color Flash SYBR Green qPCR Kit (Thermo) on IQ5 PCR Instrument (Bio-Rad Laboratories, Hercules, CA, USA). The conditions were initial denaturation at 95°C for 3 min, 30 cycles of 95°C for 30 s and 54°C for 30 s, and a final extension at 72°C for 10 min. XRCC1 and β-actin primers were designed using Primer 5.1 and synthesized by Origin Co. Ltd. (Shanghai, China) with the following sequences: XRCC1 5'-GTTCCAGCAGTGAGGAGGAT-3' and 5'-CCCCATTGTCCTGTCCTTCT-3', and β-actin 5'-CGCTGCGCTGGTCGTCGACA-3' and 5'-GTCACGCACGATTTCCCGCT-3'. The experiments were in triplicate and repeated at least three times.

Western blot analysis

Total cellular protein was extracted after homogenization in radioimmunoprecipitation assay (RIPA) buffer (Thermo). The proteins in the lysates were quantified with bicinchoninic acid (BCA) assay kit, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). The membranes were incubated in 5% skim milk powder in Tris-buffered saline/Tween-20 (TBST) at room temperature for 2 h and then probed with XRCC1 or β-actin antibody (Abcam, Cambridge, MA, USA) at 4°C overnight. The membranes were washed with TBST three times and then incubated with a secondary antibody or 1 h at the room temperature. After washing in TBST three times, the membranes were incubated with enhanced chemiluminescence (ECL) solution, exposed to X-ray films, and quantified using the Bio-Rad ChemiDocXRS instrument (Bio-Rad Inc.).

Statistical analysis

All data were summarized as mean ± SD and analyzed by SPSS 18.0 software (SPSS, Chicago, IL, USA). The comparison among groups were assessed by using the analysis of variance (ANOVA) and the least significant difference (LSD) test, while the comparisons between two groups were analyzed using Student's *t*-test. A P value < 0.05 was considered significant.

Results

Effects of XRCC1 SNPs (Arg399Gln and Arg194Trp) on NSCLC cell viability and apoptosis

Plasmids carrying XRCC1 SNPs (Arg399Gln or Arg194Trp) or control plasmids were efficiently transfected into NSCLC A549 and A549/DDP cells (Fig. 1). In A549 cells, transfection of XRCC1 wild-type plasmid led to increased cell viability compared to untransfected cells, but the viability of A549 cells transfected with XRCC1 wild-type plasmid was significantly lower than the viability of cells transfected with XRCC1 Arg399Gln and Arg194Trp after treatment with 6 μg/ml cisplatin ($^*p < 0.05$ for XRCC1 wild-type versus Arg399Gln, $^*p < 0.05$ for XRCC1 wild-type versus Arg194Trp, Fig. 2(A)). In A549/DDP cells, transfection of XRCC1 wild-type plasmid led to increased cell viability compared to untransfected cells, but the viability of A549 cells transfected with XRCC1 wild-type plasmid was significantly lower than the viability of cells transfected with XRCC1 Arg399Gln and Arg194Trp after treatment with 2, 4, and 6 μg/ml cisplatin

(*p < 0.05 for XRCC1 wild-type versus Arg399Gln, *p < 0.05 for XRCC1 wild-type versus Arg194Trp, Fig. 2(B)). Moreover, the apoptosis rate of A549/DDP cells transfected with XRCC1 wild-type plasmid was significantly higher than that of cells transfected with XRCC1 Arg399Gln and Arg194Trp (Fig. 3).

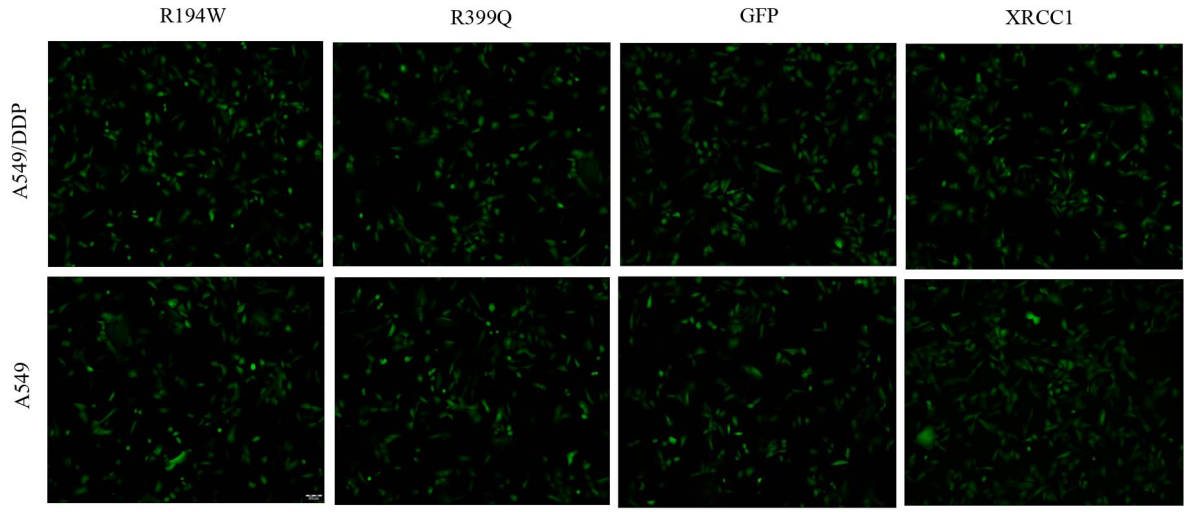

FIGURE 1. Expression of green fluorescent protein in A549 and A549/DDP cells transfected with XRCC1 SNPs. Cells were transiently transfected with plasmids carrying XRCC1 wild-type and SNPs, and then observed under a fluorescence phase contrast inverted microscope 48 h later. Magnification, × 100.

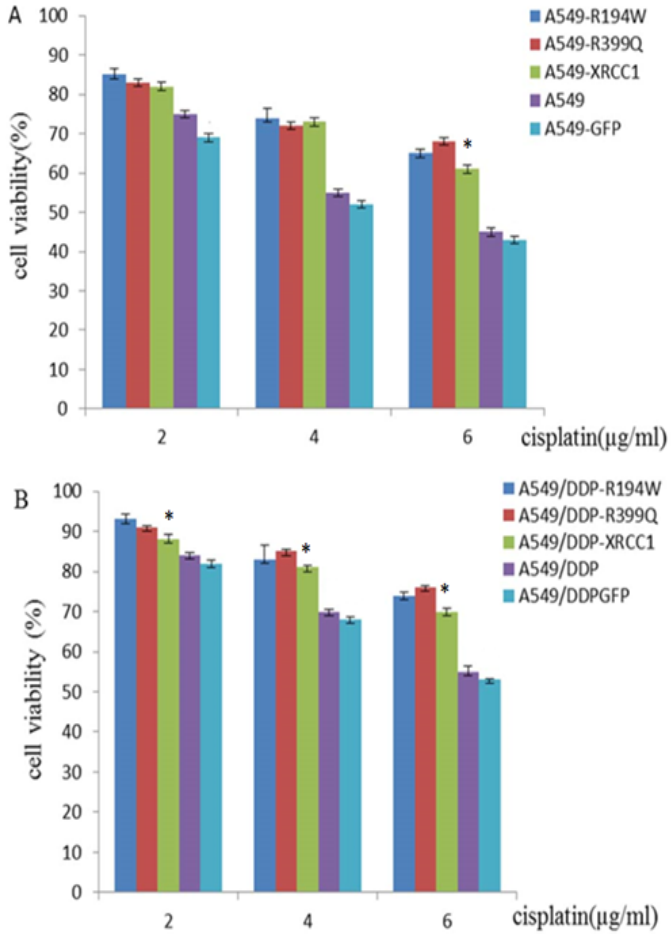

FIGURE 2. Effects of XRCC1 R399Q and R194W on NSCLC cell viability. Cells were transfected with plasmids carrying XRCC1 wild-type and SNPs, and then treated with cisplatin for additional 24 or 48 h. (A) A549 cells. (B) A549/DDP cells. *p < 0.05 for XRCC1 wild-type versus XRCC1 R399Q and R194W, respectively.

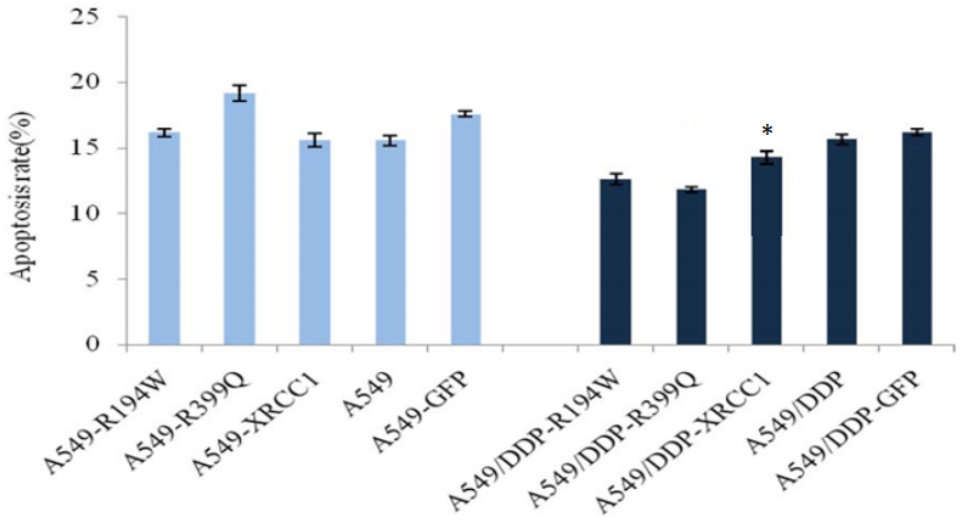

FIGURE 3. Effects of XRCC1 R399Q and R194W on NSCLC cell apoptosis. A549 or A549/DDP cells were transfected with plasmids carrying XRCC1 wild-type, XRCC1 R399Q and R194W, and then treated with 2 μg/mL cisplatin for 48 h. *$p < 0.05$ for XRCC1 wild-type versus XRCC1 R399Q and R194W, respectively.

Effects of XRCC1 Arg399Gln and Arg194Trp SNPs on XRCC1 expression in NSCLC cells

To elucidate molecular mechanisms underlying the effects of XRCC1 variants on NSCLC cells, we compared XRCC1 expression levels between control and A549 and A549/DDP cells transfected with XRCC1 Arg399Gln and Arg194Trp. We found significant upregulation of XRCC1 mRNA and protein expression in cells transfected with XRCC Arg399Gln and Arg194Trp compared with control cells (Fig. 4), respectively.

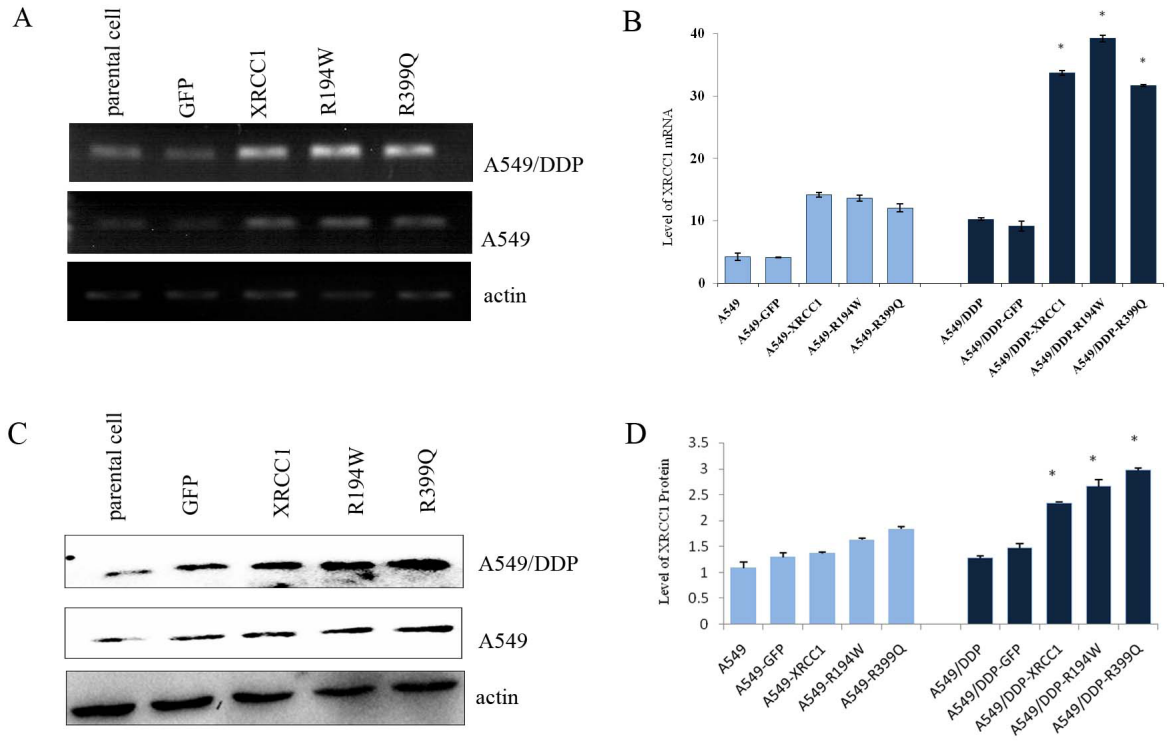

FIGURE 4. Effects of XRCC1 R399Q and Arg194Trp on XRCC1 expression in NSCLC cells. Cells were transfected with plasmids carrying XRCC1 wild-type, R399Q and R194W, and then treated with 2 μg/mL cisplatin for 48 h. (A-B) RT-PCR analysis of XRCC1 mRNA levels. (C-D) Western blot analysis of XRCC1 protein levels. *$p < 0.05$ compared with untransfected A549 and A549/DDP cells.

Discussion

XRCC1 could repair DNA single-strand break and facilitate base excision repair following DNA damage, and any changes in the scaffolding properties of XRCC1 could affect DNA repair capacity and genomic stability (London, 2015). *XRCC1* polymorphism may be a potential prognostic marker for both chemotherapy response and overall survival of cancer patients (Du *et al*., 2014). However, data on the association of XRCC1 variants with response of NSCLC patients to platinum-based chemotherapy are inconsistent, partly due to different patient populations, small sample sizes, and short follow-up (Kalikaki *et al*., 2009; Powrózek *et al*., 2016; Yao *et al*., 2009). XRCC1 protein has been shown to be overexpressed in primary and metastasized NSCLC (Kang *et al*., 2010). The mechanism of XRCC1 overexpression remains to be defined, but XRCC1 genetic variants may be implicated (Singh *et al*., 2016).

In this study, we assessed the effect of XRCC1 Arg399Gln and Arg194Trp SNPs in cisplatin-sensitive NSCLC A549 and cisplatin-resistant NSCLC A549/DDP cells. We found that transfection of XRCC1 Arg399Gln and Arg194Trp SNPs into A549 and A549/DDP cells increased cell viability after cisplatin treatment. XRCC1 variant-transfected cells also showed a reduction in apoptosis. The two identified SNPs may lead to cisplatin resistance rather than sensitivity.

A previous study suggested that XRCC1 Arg399Gln and Arg194Trp may alter XRCC1 protein levels (Zhu and Lippard, 2009). We analyzed XRCC1 mRNA and protein levels in cells transfected with XRCC1 Arg399Gln and Arg194Trp. Our data showed that both XRCC1 SNPs significantly upregulated XRCC1 expression. Our finding is consistent with a previous study demonstrating that XRCC1 transcript abundance was associated with cisplatin resistance in NSCLC cells (Weaver *et al*., 2005). However, drug resistance of cancer cells is influenced by other factors, including other DNA repair enzymes and various cell growth and apoptosis-related proteins (Bcl-2, MDR, and p53). Better understanding of the interaction between these factors may contribute to more effective cancer treatments.

In conclusion, our current study suggests that Arg399Gln and Arg194Trp SNPs in XRCC1 may change XRCC1 expression and increase the resistance of NSCLC to cisplatin. Future studies will assess the potential of XRCC1 level as a biomarker for the prediction of chemosensitivity of NSCLC.

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Conflict of Interest Statement

The authors declare no conflict of interest in this work.

References

Abdel-Rahman SZ, El-Zein RA (2000). The *399Gln* polymorphism in the DNA repair gene *XRCC1* modulates the genotoxic response induced in human lymphocytes by the tobacco-specific nitrosamine NNK. *Cancer Letters* **159**: 63-71.

Du Y, Su T, Zhao L, Tan X, Chang W, Zhang H, Cao G (2014). Associations of polymorphisms in DNA repair genes and MDR1 gene with chemotherapy response and survival of non-small cell lung cancer. *PLoS One* **9**: e99843.

Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Keep O, Castedo M, Kroemer G (2012). Molecular mechanisms of cisplatin resistance. *Oncogene* **31**: 1869-1883.

Hanssen-Bauer A, Solvang-Garten K, Akbari M, Otterlei M (2012). X-ray repair cross complementing protein 1 in base excision repair. *International Journal of Molecular Sciences* **13**: 17210-17229.

Li D, Zhou Q, Liu Y, Yang Y, Li Q (2012). DNA repair gene polymorphism associated with sensitivity of lung cancer to therapy. *Medical Oncology* **29**: 1622-1628.

Kalikaki A, Kanaki M, Vassalou H, Souglakos J, Voutsina A, Georgoulias V, Mavroudis D (2009). DNA repair gene polymorphisms predict favorable clinical outcome in advanced non-small-cell lung cancer. *Clinical Lung Cancer* **10**: 118-123.

Kang CH, Jang BG, Kim DW, Chung DH, Kim YT, Jheon S, Sung S-W, Kim JH (2010). The prognostic significance of ERCC1, BRCA1, XRCC1, and βIII-tubulin expression in patients with non-small cell lung cancer treated by platinum- and taxane-based neoadjuvant chemotherapy and surgical resection. *Lung Cancer* **68**: 478-483.

London RE (2015). The structural basis of XRCC1-mediated DNA repair. *DNA Repair* **30**: 90-103.

Moser J, Kool H, Giakzidis I, Caldecott K, Mullenders LH, Fousteri MI (2007). Sealing of chromosomal DNA nicks during nucleotide excision repair requires XRCC1 and DNA ligase IIIα in a cell-cycle-specific manner. *Molecular Cell* **27**: 311-323.

Nemer AO, Al Anazi MS, Bhat RS, Warsy AS, Babay ZA, Addar MH, Shaik J, Al-Daihan S (2018). Association between preterm birth risk and polymorphism and expression of the DNA repair genes *OGG1* and *APE1* in Saudi women. *Biocell* **42**: 1-6.

Powrózek T, Mlak R, Krawczyk P, Homa I, Ciesielka M, Kozioł P, Prendecka M, Milanowski J, Małecka-Massalska T (2016). The relationship between polymorphisms of genes regulating DNA repair or cell division and the toxicity of platinum and vinorelbine chemotherapy in advanced NSCLC patients. *Clinical and Translational Oncology* **18**: 125-131.

Singh A, Singh N, BeheraD, Sharma S (2016). Association and multiple interaction analysis among five *XRCC1* polymorphic variants in modulating lung cancer risk in North Indian population. *DNA Repair* **47**: 30-41.

Tian H, Cong P, Qi R, Gao X, Liu X, Liu H. Shan F (2017). Decreased invasion ability of hypotaurine synthesis deficient glioma cells was partially due to hypomethylation of Wnt5a promoter. *Biocell* **41**: 27-32.

Wang H, He H (2018). Characterization of multidrug-resistant *Klebsiella pneumoniae* isolated from the Chinese cobra *Naja atra* in a Beijing suburb. *Biocell* **42**: 47-54.

Weaver DA, Crawford EL, Warner KA, Elkhairi F, Khuder SA, Willey JC (2005). ABCC5, ERCC2, XPA and XRCC1 transcript abundance levels correlate with cisplatin chemoresistance in non-small cell lung cancer cell lines. *Molecular Cancer* **4**: 18.

Wen S, Fu X, Li G, He L, Zhao C, Hu X, Pan R, Guo C, Zhang X, Hu X (2016). Efficacy of tamoxifen in combination with docetaxel in patients with advanced non-small-cell lung cancer pretreated with platinum-based chemotherapy. *Anti-Cancer Drugs* **27**: 447-456.

Yao CY, Huang XE, Li C, Shen HB, Shi MQ, Feng JF, Pan LX, Tang JH (2009). Lack of influence of XRCC1 and XPD gene polymorphisms on outcome of platinum-based chemotherapy for advanced non small cell lung cancers. *Asian Pacific Journal of Cancer Prevention* **10**: 859-864.

Yuan P, Liu L, Wu C, Zhong R, Yu D, Wu J, Xu Y, Nie S, Miao X, Sun Y, Xu B, Lin D (2010). No association between *XRCC1* polymorphisms and survival in non-small-cell lung cancer patients treated with platinum-based chemotherapy. *Cancer Biology & Therapy* **10**: 854-859.

Zhu G, Lippard SJ (2009). Photoaffinity labeling reveals nuclear proteins that uniquely recognize cisplatin-DNA interstrand cross-links. *Biochemistry* **48**: 4916-4925.