Role of *XRCC1* gene polymorphisms in non-small cell lung cancer cisplatin-based chemotherapy, and their effect on clinical and pathological characteristics

H.F. Liu, J.S. Liu, J.H. Deng and R.R. Wu

Department of Oncology, Ganzhou People's Hospital, Ganzhou, China

Corresponding author: J.S. Liu
E-mail: liujsgz@163.com

Genet. Mol. Res. 15 (4): gmr15049084
Received August 17, 2016
Accepted October 19, 2016
Published December 23, 2016
DOI http://dx.doi.org/10.4238/gmr15049084

ABSTRACT. Non-small cell lung cancer (NSCLC) is the most common cancer globally. The XRCC1 protein interacts with ligase and poly(ADP-ribose) polymerase to repair cisplatin-induced DNA damage. The authors of previous studies have reported *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and advanced NSCLC prognosis, but the results are inconclusive. We investigated the association between clinical outcome and *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms in advanced NSCLC patients treated with cisplatin. We recruited 252 patients with advanced NSCLC (TNM stages: IIIB and IV) and used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to genotype the polymorphisms. Patients with the TT genotype of *XRCC1* Arg194Trp showed a significantly better response to chemotherapy than those with the CC genotype. The GA+AA genotype of Arg194Trp

was correlated with better response to chemotherapy than the wild-type form. The TT genotype of Arg194Trp was associated with longer survival time than the CC genotype. The TT genotype of Arg194Trp was correlated with lower risk of death from all causes than the CC genotype. The Arg194Trp polymorphisms interacted with squamous cell carcinoma and affected overall survival of advanced NSCLC. However, there was no association between Arg399Gln and Arg280His polymorphisms and response to cisplatin-based chemotherapy and overall survival in advanced NSCLC. The results suggest that the TT genotype of Arg194Trp is significantly associated with better response to chemotherapy and longer overall survival of advanced NSCLC patients than the wild-type form. Our investigation offers insight into the influence of *XRCC1* gene polymorphisms on the treatment outcome of advanced NSCLC.

Key words: *XRCC1*; Arg399Gln; Arg280His; Arg194Trp; Cisplatin; Advanced NSCLC

INTRODUCTION

Lung cancer has been the most common cancer in the world for several decades. The statistics have shown that morbidity due to lung cancer is high and the 5-year survival rate is approximately 14% (Henley et al., 2014). Non-small cell lung cancer (NSCLC) accounts for 80-85% of lung cancer cases and at least 51% of lung cancer patients are diagnosed with metastatic disease. Although many treatment methods, including immune therapy, medication therapy, and surgery, have played crucial roles in NSCLC treatment (Molina et al., 2008; Maemondo et al., 2010), the results remain unsatisfactory owing to the poorly understood and complicated pathogenesis of NSCLC. The TNM stage is the main influence on the prognosis of NSCLC, but NSCLC patients with the same TNM stage show different treatment outcomes, even when they receive the same anti-cancer therapy (Woodard et al., 2016). Therefore, it is thought that hereditary factors may contribute to the prognosis of NSCLC.

The X-ray repair cross-complementing group 1 gene (*XRCC1*) plays a major role in the base excision repair pathway (Lindahl and Wood, 1999). The XRCC1 protein interacts with ligase and poly(ADP-ribose) polymerase to efficiently repair DNA damage, including cisplatin-induced damage (Zhu and Lippard, 2009). There are a number of single nucleotide polymorphisms in *XRCC1*, three of which [Arg399Gln (rs25487, G/A), Arg280His (rs25489, G/A), and Arg194Trp (rs1799782, C/T)] have been widely studied (Ladiges, 2006). The authors of previous studies have reported that the Arg399Gln, Arg280His, and Arg194Trp variants are associated with lung cancer, sufferers of which experience smoking-induced genotoxic damage, implying that the polymorphisms of *XRCC1* may alter the phenotype of the XRCC1 protein, which influences the response to cisplatin-based chemotherapy in advanced NSCLC (Schneider et al., 2005).

The authors of previous studies have reported the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and the prognosis of advanced NSCLC, but the results are inconclusive (Yuan et al., 2006; Wang et al., 2008; Ke et al., 2012; Han et al., 2015). To date, no research has been conducted on the interaction between *XRCC1* polymorphisms and clinical

and pathological characteristics in the treatment outcome of advanced NSCLC. Therefore, we investigated the relationship between the clinical outcome and the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms in advanced NSCLC patients subjected to cisplatin-based chemotherapy.

MATERIAL AND METHODS

Study subjects

A total of 252 patients with advanced NSCLC (TNM stages: IIIB and IV) were recruited from the Department of Oncology at Ganzhou People's Hospital between January 2010 and December 2012. All cases were histopathologically confirmed by two pathologists. The inclusion criteria were as follows: the patients had not received any chemotherapy or radiotherapy before enrollment in our study, had satisfactory hematology results and normal renal and liver function, and had an Eastern Cooperative Oncology Group performance status of 0 or 1. Patients were excluded from this study if they had a serious concomitant systemic disorder, were unable to receive chemotherapy, had brain metastasis with symptoms, or had other serious diseases that may have influenced the safety of the chemotherapy.

All patients received cisplatin-based chemotherapy after enrolling in the study. Patients with advanced NSCLC received at least two cycles of chemotherapy, and the chemotherapy treatment was repeated at three intervals for less than six cycles. The chemotherapy was suspended until disease progression or unacceptable toxicity. If patients presented three grades of non-hematology toxicity, four grades of hematology toxicity, febrile neutropenia, or infection, the dosage of chemotherapy treatment was decreased by 25% in the subsequent cycle.

The response to chemotherapy was evaluated using the Response Evaluation Criteria in Solid Tumors (Duffaud and Therasse, 2000). Patients who showed a complete response or a partial response to chemotherapy were regarded as good responders, and those who presented stable disease or progressive disease to chemotherapy were considered poor responders. The study was carried out with the permission of the Institutional Review Board of the Department of Oncology at Ganzhou People's Hospital. Written informed consent was obtained from all participants.

All patients were followed up by return visit or telephone enquiry between January 2010 and December 2014. Up to December 2014, the patients were followed up for 6-60 months with a median follow-up time of 17 months and an average follow-up time of 16.52 ± 10.35 months. A total of 238 cases were followed up, whereas 14 patients were lost to follow-up.

DNA extraction and genotyping

All subjects were asked to provide 5-mL peripheral venous blood samples before receiving cisplatin-based chemotherapy. DNA was extracted from the peripheral blood samples, which were collected from patients using a TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to the manufacturer instructions. The genotyping of *XRCC1* Arg399Gln, Arg280His, and Arg194Trp was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzymes (Table 1). The PCR conditions were as follows: initial denaturation at 95°C for 5 min; 30 cycles of denaturation at 94°C for 60 s, annealing at 60°C for 60 s, and extension at 72°C for 60 s; and a final extension

at 72°C for 10 min. The digestion products were separated by electrophoresis on ethidium bromide-stained agarose gel, and visualized under UV light.

Table 1. Primers and restriction enzymes for the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms.

Gene polymorphism	Primers	Length of digested fragment	Restriction enzyme
XRCC1 Arg399Gln	5'-CCAGTGGTGCTAACCTAATC-3'	201 bp	*Msp*I
	5'-CACTCAGCACCACTACCACA-3'		
	5'-CTATCATCTCCTGGCCCCC-3'		
XRCC1 Arg280His	5'-TTGTGCTTTCTCTGTGTCCA-3'	615 bp	*Rsa*I
	5'-TCCTCCAGCCTTTTCTGATA-3'		
XRCC1 Arg194Trp	5'-GCCCGCTCTGGATTATACG-3'	485 bp	*Pvu*II
	5'-CTATCATCTCCTGGCCCCC-3'		

Statistical analysis

The SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The measured data are reported as means ± standard deviation or frequency (N) and percentage (%). Multiple regression analysis was used to assess the association between the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp gene polymorphisms and the response to chemotherapy in advanced NSCLC, and the results were assessed by odds ratios (ORs) and their corresponding 95% confidence intervals (95%CIs). Survival analysis was performed using the log-rank test. Multivariate analysis was carried out to assess the association between the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and the overall survival of NSCLC using the Cox proportional hazard model, and the results were assessed by hazard ratios (HRs) and their 95%CIs. A P value of < 0.05 was considered to indicate a statistically significant result.

RESULTS

The clinical and pathological characteristics of patients with advanced NSCLC are summarized in Table 2. The mean age of the included NSCLC patients was 56.31 ± 12.55 years. There were 104 males and 148 females. Of the 252 advanced NSCLC patients, 190 (75.40%) smoked, 149 (59.13%) exhibited adenocarcinoma, 111 (44.05%) showed good differentiation, 141 (55.95%) presented moderate or poor differentiation, 183 (72.62%) were at TNM stage IIIB, 69 (27.38%) were at TNM stage IV, and 76 (30.16%) demonstrated a good response to chemotherapy.

At the end of the follow-up period, 76 (30.16%) patients showed a good response to chemotherapy. Logistic regression analysis revealed that patients carrying the TT genotype of *XRCC1* Arg194Trp showed a significantly better response to chemotherapy than those with the CC genotype (OR = 4.28, 95%CI = 1.62-11.29, P = 0.001). Moreover, the GA+AA genotype of *XRCC1* Arg194Trp was correlated with better response to chemotherapy compared with the wild-type form (OR = 1.97, 95%CI = 1.09-3.59) (Table 3). However, we did not observe an association between *XRCC1* Arg399Gln and Arg280His polymorphisms and response to cisplatin-based chemotherapy in advanced NSCLC.

A total of 216 patients died during the follow-up period by the end of December 2014, and the 5-year survival rate of advanced NSCLC patients was calculated to be 14.29%. The TT genotype of *XRCC1* Arg194Trp was associated with longer survival time compared with the CC genotype (19.43 months *vs.* 16.50 months, P value for the log-rank test = 0.02) (Table 4).

Table 2. Clinical and pathological characteristics of patients with advanced non-small cell lung cancer (NSCLC).

Variables	Patients (N)	%
Gender		
Male	104	41.27
Female	148	58.73
Age, years		
<60	113	44.84
≥60	139	55.16
Smoking habit		
No	62	24.60
Yes	190	75.40
Pathological type		
Squamous cell carcinoma	103	40.87
Adenocarcinoma	149	59.13
Degree of differentiation		
Well-differentiated	111	44.05
Moderately or poorly differentiated	141	55.95
TNM stage		
IIIB	183	72.62
IV	69	27.38
Response to chemotherapy		
Good	76	30.16
Poor	176	69.84

Table 3. Association between the*XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and response to chemotherapy in advanced non-small cell lung cancer (NSCLC) patients.

XRCC1	Patients	%	Good response	%	Poor response	%	OR (95%CI)[1]	P value
Arg399Gln								
GG	112	42.75	31	40.79	81	45.25	1.0 (Ref.)	-
GA	107	40.84	32	42.11	75	41.90	1.11 (0.60-2.09)	0.72
AA	33	12.60	13	17.11	20	11.17	1.70 (0.69-4.09)	0.2
GA+AA	140	53.44	45	59.21	95	53.07	1.24 (0.69-2.22)	0.44
Arg280His								
AA	165	62.98	47	61.84	118	65.92	1.0 (Ref.)	-
AG	66	25.19	20	26.32	46	25.70	1.09 (0.55-2.12)	0.78
GG	21	8.02	9	11.84	12	6.70	1.88 (0.65-5.22)	0.18
AG+GG	87	33.21	29	38.16	58	32.40	1.26 (0.69-2.27)	0.43
Arg194Trp								
CC	115	43.89	26	34.21	89	49.72	1.0 (Ref.)	-
CT	110	41.98	35	46.05	75	41.90	1.60 (0.85-3.03)	0.12
TT	27	10.31	15	19.74	12	6.70	4.28 (1.62-11.29)	0.001
CT+TT	137	52.29	50	65.79	87	48.60	1.97 (1.09-3.59)	0.02

[1]Adjusted for age, gender, smoking habit, pathological type, degree of differentiation, and TNM stage.

According to the Cox proportional hazards model, the TT genotype of *XRCC1* Arg194Trp was correlated with a lower risk of death from all causes compared with the CC genotype (HR = 0.17, 95%CI = 0.06-0.50, P value < 0.001). However, the *XRCC1* Arg399Gln and Arg280His gene polymorphisms did not influence the overall survival of advanced NSCLC patients (P > 0.05). We also investigated the interaction between *XRCC1* Arg194Trp polymorphisms and clinical and pathological characteristics in the overall survival of advanced NSCLC (Table 5). *XRCC1* Arg194Trp polymorphisms interacted with squamous cell carcinoma and affected the overall survival of advanced NSCLC (correlation coefficient = 0.162, P = 0.04). We found that the *XRCC1* Arg194Trp polymorphisms did not affect squamous cell carcinoma, adenocarcinoma, degree of differentiation, or TNM stage in the overall survival of advanced NSCLC.

Table 4. Cox regression analysis of the association between the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and overall survival of advanced non-small cell lung cancer (NSCLC) patients.

Genotypes	Death	%	Alive	%	P value for log-rank test	Median survival time (months)	HR (95%CI)[1]	P value
Arg399Gln								
GG	93	43.06	19	52.78		17.15	1.0 (Ref.)	-
GA	96	44.44	11	30.56		15.82	1.78 (0.76-4.38)	0.15
AA	27	12.50	6	16.67		18.15	0.92 (0.31-3.10)	0.87
GA+AA	123	56.94	17	47.22	0.28	16.43	1.48 (0.68-3.21)	0.28
Arg280His								
AA	140	64.81	25	69.44		17.37	1.0 (Ref.)	-
AG	59	27.31	7	19.44		16.62	1.51 (0.59-4.35)	0.37
GG	17	7.87	4	11.11		18.90	0.76 (0.22-3.36)	0.64
AG+GG	76	35.19	11	30.56	0.16	17.04	1.22 (0.54-2.93)	0.59
Arg194Trp								
CC	101	46.76	14	38.89		16.50	1.0 (Ref.)	-
CT	100	46.30	10	27.78		15.15	1.39 (0.54-3.66)	0.45
TT	15	6.94	12	33.33		19.43	0.17 (0.06-0.50)	<0.001
CT+TT	115	53.24	22	61.11	0.02	18.25	0.72 (0.32-1.57)	0.38

[1]Adjusted for age, gender, smoking habit, pathological type, degree of differentiation, and TNM stage.

Table 5. Interaction between the *XRCC1* Arg194Trp polymorphisms and clinical and pathological characteristics in overall survival of advanced non-small cell lung cancer (NSCLC) patients.

Characteristics	Correlation coefficient	P value
Squamous cell carcinoma	0.162	0.04
Adenocarcinoma	0.073	0.27
Degree of differentiation	0.061	0.32
TNM stage	0.047	0.45

DISCUSSION

In this study, we evaluated the association between the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and the response to chemotherapy and overall survival in advanced NSCLC patients in a Chinese population. The results of our study indicated that the TT genotype of *XRCC1* Arg194Trp was associated with a good response to chemotherapy, and was correlated with high overall survival of NSCLC.

The 194Trp codon of *XRCC1* is located in a highly conserved hydrophobic linker region between its DNA polymerase domain and the poly (ADP-ribose) polymerase-interacting domains, so the substitution from Arg to Trp could alter the interaction between *XRCC1* and either or both of these DNA repair proteins within the base excision repair complex (Vidal et al., 2001). Previous studies have indicated that individuals carrying the T allele exhibit a significantly higher frequency of chromatid exchanges compared with those with the C allele (Au et al., 2003). DNA repair requires an undamaged sister chromatid, and the T allele of *XRCC1* Arg194Trp is associated with lower DNA repair activity, which may increase the response to chemotherapy in cancer patients.

The authors of previous studies have reported an association between the *XRCC1* Arg194Trp polymorphism and the prognosis of several kinds of cancer treated using cisplatin-based chemotherapy (Wang et al., 2008; Miao et al., 2012; Li and Li, 2013; Cao et al., 2014; Yang and Zhao, 2015). Miao et al. (2012) conducted a study on 195 Chinese patients with primary advanced ovarian cancer, and reported that the TT genotype of *XRCC1* conferred a significant risk of death from ovarian cancer compared with the CC genotype. Wang et al. (2008)

conducted a study to assess the association between the *XRCC1* Arg194Trp polymorphism and the prognosis of lung cancer, and found no statistically significant association between the polymorphism and the prognosis of lung cancer. Yang and Zhao (2015) conducted a study on 118 hepatocellular carcinoma patients and reported that the *XRCC1* Arg194Trp polymorphism is involved in the cisplatin resistance of hepatocellular carcinoma cells. Cao et al. (2014) conducted a systematic review to assess the *XRCC1* gene polymorphisms and treatment outcome of advanced gastric cancer, and reported that the *XRCC1* Arg194Trp polymorphism contributes to the treatment outcome of advanced gastric cancer.

Only four studies have indicated an association between the *XRCC1* Arg194Trp polymorphism and the prognosis of NSCLC patients receiving cisplatin-based chemotherapy (Yuan et al., 2006; Wang et al., 2008; Ke et al., 2012; Han et al., 2015). Yuan et al. (2006) conducted a study on 200 patients with advanced NSCLC who received platinum-based chemotherapy; the study suggested that the *XRCC1* Arg194Trp polymorphism may be associated with clinical responses to platinum-based chemotherapy in advanced NSCLC. Wang et al. (2008) conducted a study in a Chinese population, and did not find a significant correlation between *XRCC1* Arg194Trp and advanced NSCLC treated using cisplatin-based chemotherapy. A study by Ke et al. (2012) conducted in a Chinese population indicated that *XRCC1* Arg194Trp played a role in modifying the effect of platinum-based chemotherapy in NSCLC patients. Han et al. (2015) conducted a study on 325 advanced NSCLC patients and found that *XRCC1* Arg194Trp influences the response to cisplatin-based chemotherapy in advanced NSCLC. In the current study, we found that the TT genotype of the *XRCC1* Arg194Trp polymorphism was associated with a better treatment outcome in advanced NSCLC compared with the CC genotype. Further studies are required to confirm our findings.

There were several limitations to our study: the advanced NSCLC patients were selected from one hospital only, which may have resulted in selection bias. The sample size used for the analysis of the association between the *XRCC1* Arg399Gln, Arg280His, and Arg194Trp polymorphisms and advanced NSCLC survival was relatively small. Studies with larger sample sizes should be carried out to confirm our results.

In conclusion, the results of our study indicated that the TT genotype of the *XRCC1* Arg194Trp polymorphism was significantly associated with better response to chemotherapy and longer overall survival of advanced NSCLC patients than the wild-type form. The *XRCC1* Arg194Trp polymorphisms interacted with squamous cell carcinoma and affected the overall survival of advanced NSCLC patients. Our investigation offers insight into the influence of *XRCC1* gene polymorphisms on the treatment outcome of advanced NSCLC. Further prospective studies with larger sample sizes are required to validate this association.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Au WW, Salama SA and Sierra-Torres CH (2003). Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. *Environ. Health Perspect.* 111: 1843-1850. http://dx.doi.org/10.1289/ehp.6632

Cao Z, Song J, Wang J, Guo X, et al. (2014). Association between polymorphisms in XRCC1 gene and treatment outcomes of patients with advanced gastric cancer: a systematic review and meta-analysis. *PLoS One* 9: e85357. http://dx.doi.org/10.1371/journal.pone.0085357

Duffaud F and Therasse P (2000). [New guidelines to evaluate the response to treatment in solid tumors]. *Bull. Cancer* 87: 881-886.

Han B, Guo Z, Ma Y, Kang S, et al. (2015). Association of GSTP1 and XRCC1 gene polymorphisms with clinical outcome of advanced non-small cell lung cancer patients with cisplatin-based chemotherapy. *Int. J. Clin. Exp. Pathol.* 8: 4113-4119.

Henley SJ, Richards TB, Underwood JM, Eheman CR, et al.; Centers for Disease Control and Prevention (CDC) (2014). Lung cancer incidence trends among men and women--United States, 2005-2009. *MMWR Morb. Mortal. Wkly. Rep.* 63: 1-5.

Ke HG, Li J, Shen Y, You QS, et al. (2012). Prognostic significance of GSTP1, XRCC1 and XRCC3 polymorphisms in non-small cell lung cancer patients. *Asian Pac. J. Cancer Prev.* 13: 4413-4416. http://dx.doi.org/10.7314/APJCP.2012.13.9.4413

Ladiges WC (2006). Mouse models of XRCC1 DNA repair polymorphisms and cancer. *Oncogene* 25: 1612-1619. http://dx.doi.org/10.1038/sj.onc.1209370

Li K and Li W (2013). Association between polymorphisms of XRCC1 and ADPRT genes and ovarian cancer survival with platinum-based chemotherapy in Chinese population. *Mol. Cell. Biochem.* 372: 27-33. http://dx.doi.org/10.1007/s11010-012-1442-4

Lindahl T and Wood RD (1999). Quality control by DNA repair. *Science* 286: 1897-1905. http://dx.doi.org/10.1126/science.286.5446.1897

Maemondo M, Inoue A, Kobayashi K, Sugawara S, et al.; North-East Japan Study Group (2010). Gefitinib or chemotherapy for non-small-cell lung cancer with mutated EGFR. *N. Engl. J. Med.* 362: 2380-2388. http://dx.doi.org/10.1056/NEJMoa0909530

Miao J, Zhang X, Tang QL, Wang XY, et al. (2012). Prediction value of XRCC 1 gene polymorphism on the survival of ovarian cancer treated by adjuvant chemotherapy. *Asian Pac. J. Cancer Prev.* 13: 5007-5010. http://dx.doi.org/10.7314/APJCP.2012.13.10.5007

Molina JR, Yang P, Cassivi SD, Schild SE, et al. (2008). Non-small cell lung cancer: epidemiology, risk factors, treatment, and survivorship. *Mayo Clin. Proc.* 83: 584-594. http://dx.doi.org/10.1016/S0025-6196(11)60735-0

Schneider J, Classen V, Bernges U and Philipp M (2005). XRCC1 polymorphism and lung cancer risk in relation to tobacco smoking. *Int. J. Mol. Med.* 16: 709-716.

Vidal AE, Boiteux S, Hickson ID and Radicella JP (2001). XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *EMBO J.* 20: 6530-6539. http://dx.doi.org/10.1093/emboj/20.22.6530

Wang Z, Xu B, Lin D, Tan W, et al. (2008). XRCC1 polymorphisms and severe toxicity in lung cancer patients treated with cisplatin-based chemotherapy in Chinese population. *Lung Cancer* 62: 99-104. http://dx.doi.org/10.1016/j.lungcan.2008.02.019

Woodard GA, Jones KD and Jablons DM (2016). Lung cancer staging and prognosis. *Cancer Treat. Res.* 170: 47-75. http://dx.doi.org/10.1007/978-3-319-40389-2_3

Yang Z and Zhao J (2015). Effect of APE1 and XRCC1 gene polymorphism on susceptibility to hepatocellular carcinoma and sensitivity to cisplatin. *Int. J. Clin. Exp. Med.* 8: 9931-9936.

Yuan P, Miao XP, Zhang XM, Wang ZH, et al. (2006). [XRCC1 and XPD genetic polymorphisms predict clinical responses to platinum-based chemotherapy in advanced non-small cell lung cancer]. *Zhonghua Zhong Liu Za Zhi* 28: 196-199.

Zhu G and Lippard SJ (2009). Photoaffinity labeling reveals nuclear proteins that uniquely recognize cisplatin-DNA interstrand cross-links. *Biochemistry* 48: 4916-4925. http://dx.doi.org/10.1021/bi900389b