8-OHdG repair is associated with platinum sensitivity in high-grade serous ovarian carcinoma

Jing Ni, Yan Wang, Qian Zhao, Xia Xu, Hong-Yuan Gu, Xian-Zhong Cheng, Rui Zhou, Yan Li, Wen-Wen Guo, Xiao-Xiang Chen

1 Department of Gynecologic Oncology, The Affiliated Cancer Hospital of Nanjing Medical University, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, 210009 Nanjing, Jiangsu, China
2 Department of Pathology, The Second Affiliated Hospital of Nanjing Medical University, 210011 Nanjing, Jiangsu, China
3 Department of Chemotherapy, Jiangsu Cancer Hospital, Jiangsu Institute of Cancer Research, The Affiliated Cancer Hospital of Nanjing Medical University, 210009 Nanjing, Jiangsu, China
4 Nanjing Gaochun People’s Hospital, 211300 Nanjing, Jiangsu, China
5 The Medical College of Yangzhou University, 225009 Yangzhou, Jiangsu, China

*Correspondence: cxxxxcyd@gmail.com (Xiao-Xiang Chen)
† These authors contributed equally.

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8-OHdG is a well-characterized oxidized base that can incorrectly incorporate adenines opposite lesion of 8-OHdG and trigger G:C to T:A in genomic DNA. Oxidized DNA bases can activate the repair system after DNA damage in order to maintain genomic stability, such as the base excision repair (BER) system. DNA glycosylases, including hOGG1, MUTYH, and MTH1, are associated with the BER system. These enzymes cooperate with TP53 to repair DNA oxidative damage, excise 8-OHdG located on the damaged DNA strands and protect cells from the mutagenic effects of 8-OHdG. However, reduced ability to repair damaged DNA can promote gene mutation. Most of the genetic variations in BER system-induced genomic instability are closely related to tumor susceptibility. It is unclear whether these variations are associated with drug resistance, however it is assumed that variants of the 8-OHdG repair-related gene may lead to repair dysfunction and the accumulation of oxidative DNA damage. This in turn could eventually induce platinum resistance based on previous findings on the associations of 8-OHdG repair-related gene polymorphisms with oxidative DNA damage in gastric cancer, breast cancer, ovarian cancer and diabetes [9–18].

Keywords
8-OHdG, Genetic polymorphism; Platinum sensitivity, High-grade serous ovarian carcinoma

1. Introduction

Epithelial ovarian cancer (EOC) is the most common lethal gynecologic malignancy worldwide [1–3]. Approximately 50% of EOC patients are characterized as high-grade serous adenocarcinoma, for which the standard treatment is cytoreductive surgery and platinum-based chemotherapy [4, 5]. The majority of HG-SOCs initially respond well to platinum-based therapy, with only about 14% HG-SOCs being platinum-refractory cases [6, 7]. However, poor outcome with a 5-year survival rate of < 30% is due to acquired resistance to platinum therapy. Cross-linking between DNA strands and platinum induces subsequent strand breaks which then leads to tumor cell destruction. Therefore, tumor cells that acquire platinum resistance are able to overcome this DNA damaging process. Platinum resistance is considered to result from the presence of highly resistant mutants at low frequencies before these mutants are exposed to platinum [8].
Table 1. Host and clinical characteristics of high-grade serous ovarian carcinoma patients and controls.

| Characteristic                              | Cases            | Controls         | P-value |
|--------------------------------------------|------------------|------------------|---------|
| Age in years, median (range)               | 61.5 (36–83)     | 59.2 (33–80)     | 0.55    |
| BMI, mean (SD)                             | 25.5 (3.42)      | 23.7 (3.54)      | 0.49    |
| Menopausal status (%)                      |                  |                  |         |
| Postmenopausal                             | 256              | 495              | 0.41    |
| Premenopausal                              | 94               | 205              |         |
| Baseline CA-125 level, median (range, U/mL)| 900 (32–28000)   |                  |         |
| FIGO stage (%)                             |                  |                  |         |
| I                                          | 52 (14.9)        |                  |         |
| II                                         | 17 (4.9)         |                  |         |
| III                                        | 180 (51.4)       |                  |         |
| IV                                         | 87 (24.9)        |                  |         |
| Unknown                                    | 14 (4.0)         |                  |         |
| Surgical residual (%)                      |                  |                  |         |
| Optimal                                    | 240 (68.6)       |                  |         |
| Sub-optimal                                | 98 (28.0)        |                  |         |
| Unknown                                    | 12 (3.4)         |                  |         |
| Neo-adjuvant chemotherapy (%)              | 213 (60.9)       |                  |         |
| Refractory and Recurrence                  |                  |                  |         |
| Refractory (%)                             | 44 (12.6)        |                  |         |
| Platinum-sensitive recurrent (%)           | 46 (13.7)        |                  |         |
| Platinum-resistant recurrent (%)           | 195 (55.7)       |                  |         |

BMI, Body Mass Index, FIGO, the International Federation of Gynecology and Obstetrics. Platinum-resistant recurrent was defined as tumor progression during or within six months after completion of prior platinum therapy [23, 24].

This case-control study was performed to determine the relationship between polymorphisms in the 8-OHdG repair-related gene and HG-SOC risk and survival. We examined the correlation of polymorphisms with 8-OHdG concentration and platinum sensitivity in a Chinese population cohort with HG-SOC. We also assessed 8-OHdG concentration in leukocyte DNA in order to examine the association between the level of oxidative damage and survival among HG-SOC patients.

2. Materials and methods
2.1 Subjects
All patients (cases, n = 350) were diagnosed with sporadic HG-SOC and given primary treatment in the Department of Gynecologic Oncology at Jiangsu Cancer Hospital (Nanjing, Jiangsu province, China) between January 2003 and December 2013. Healthy women in the age-matched control group (n = 700) were recruited from volunteers undergoing health checks in the same region. The allele frequencies of OGG1c.977C > G (rs1052133), MTH1 c.247G > A (rs4866), MUTYH c.972G > C (rs3219489) and AluYb8MUTYH (rs10527342), TP53 codon 72 Arg/Pro (rs1042522) and 16-bp Ins/Del were investigated. Clinical data including follow up were collected in all subjects (Table 1).

2.2 High-resolution melting analysis
BER genetic variants (OGG1 c.977C > G, MTH1 c.247G > A and MUTYH c.972G > C) were genotyped by dsDNA dye LC Green and HRM analysis. The PCR primers were designed by LightScanner primer design software (Idaho Technology) as mentioned previously [9–11, 13–18] and are listed in Table 2.

2.3 AluYb8MUTYH polymorphism assay
The PCR primers are listed in Table 2. Products were run on 1% agarose gels (Invitrogen, Carlsbad, CA, USA) as described previously [18]. The AluYb8MUTYH genotypes were classified into 3 groups based upon absence or presence of the fragment, including homozygous absence as follows: only 500 bp products, absence/absence, A/A; homozygous presence of the variant (only 826 bp products, presence/presence, P/P); heterozygote (500 bp and 826 bp products, absence/presence, A/P).

2.4 TP53 codon 72 Arg/Pro (rs1042522) and 16-bp Ins/Del polymorphism assay
The codon 72 polymorphism was examined by direct sequencing. PCR primers are also listed in Table 2. The PCR thermal cycling conditions were as previously described [19].

2.5 Evaluation of 8-OHdG concentration in genomic DNA from blood cells
100 DNA samples from the HG-SOCs were randomly recruited by the method reported previously [9, 14, 15]. Briefly, an ELISA kit (Highly Sensitive 8-OHdG Check, JaICA, Fukuroi, Shizuoka, Japan) was used to assay the 8-OHdG concentration and converted to 8-OHdG/10^6 dG based on Halliwell.
Table 2. Sequences of PCR Primers used for genotyping.

| Polymorphisms          | Primer sequence (5′-3′) | Annealing temperature (°C) | Product length (bp) |
|------------------------|------------------------|----------------------------|---------------------|
| rs1052133: (OGG1 c.977C > G) | F: 5′-actgtcactagtctcaacag-3′ R: 5′-ggaaggtgcttggggaat-3′ | 55                      | 200                 |
| rs4866: (MTH1 c.247G > A)  | F: 5′-gagcggtctgacagtgga-3′ R: 5′-tggcactcagagatggtttg-3′ | 58                      | 168                 |
| rs3219489: (MUTYH c.972G > C) | F: 5′-cccattccagttcttcctct-3′ R: 5′-cctttctggggaagttgacc-3′ | 58                      | 208                 |
| rs10527342: (AluYb8MUTYH) | F: 5′-tcttgacctggagaccttcc-3′ R: 5′-agctgcttcctccaaacagc-3′ | 60                      | 500 or 826          |
| rs1042522: (TP53 Arg72Pro) | F: 5′-gacctggtcctctgactgctct-3′ R: 5′-tgacaggaagccaaagggtgaagag-3′ | 59                      | 430                 |
| TP5316-bp Ins/Del       | F: 5′-cgtctctgaaggcccaagggcttg-3′ R: 5′-aaagacagctgacagacaccgctc-3′ | 59                      | 102 or 118          |

2.6 8-OHdG expression assay in tissues

Immunohistochemical (IHC) staining was performed to evaluate 8-OHdG expression in HG-SOC samples within tissue microarray. Pathologically confirmed sections from 16 platinum-resistant and 24 platinum-sensitive recurrent cases were obtained from Jiangsu Cancer Hospital. Briefly, 3–10 μm thick sections containing representative tumor samples were cut from paraffin blocks and mounted on slides. IHC staining was performed by the avidin-biotin peroxidase system. 8-OHdG staining was semi-quantitatively assessed by color intensity and the percentage of staining cells. The color intensity was stratified with three scales, and the percentage of staining cells was judged with two scales. The samples was subsequently classified as strong positive (+++), moderate positive (++), weak positive (+), and negative (-). Negative control slides were included in the assay.

2.7 Statistical analysis

Statistical analyses were carried out using SPSS, Version 15.0. Descriptive statistics included mean ± SD for continuous data and percentages for categorical data. Chi-square tests were performed to compare the genotype and allelic frequencies between cases and controls. Odds ratios (OR) were shown with 95% confidence intervals (CIs). Variables were separately compared among cases with different genotypes through ANOVA and post hoc analysis. Since the 8-OHdG concentration in leukocyte DNA was positively skewed, a natural logarithm transformation was used to normalize the distribution prior to analysis. In all analyses, P < 0.05 was considered statistically significant. Cox proportional hazards models were used to evaluate the correlation between OS and clinicopathological characteristics, including the 8-OHdG concentration. The Kaplan-Meier method was used to calculate the log-rank test for observed survival (OS) and progression-free survival (PFS). Values were stratified in terms of 8-OHdG alteration.

2.8 Ethical approval

Institutional Ethics Committee of Nanjing Medical University (2020-108) approved this retrospective study. The requirement for informed consent was waived.

3. Results

3.1 Genotyping of 8-OHdG repair-related gene polymorphisms in HG-SOC patients

The genotype frequencies for OGG1 c.977C > G, MTH1 c.247G > A, MUTYH c.972G > C, AluYb8MUTYH, TP53 codon 72 Arg/Pro and the 16-bp Ins/Del associated with HG-SOC are shown in Table 3. The distribution of these polymorphisms in the control group was not statistically different from the Hardy-Weinberg equilibrium (P > 0.05 for all). The genotypes for OGG1 c.977 C > G (CC, CG, and GG) in HG-SOCs were distributed differently from healthy controls (P = 0.04). Furthermore, the frequency of the OGG1 c.977 GG genotype in HG-SOCs (36.0%) was statistically higher than in the controls (29.7%). The age-adjusted OR [cases: controls] for GG was 1.33 (95% CI: 1.01–1.75; P = 0.04). For the c.247G > A in MTH1, the frequency of heterozygous MTH1 c.247G > A was 6.0% among cases and 7.7% among controls (P = 0.31), while the homozygote was not detected. Thus, the MTH1 c.247G > A polymorphism was not included in further analysis. The distribution of the three genotypes, namely, CC, CG, and GG, and the allele frequencies of C and G in MUTYH c.972G > C were not different between HG-SOCs (cases) and the healthy controls (P > 0.05). For the 16-bp Ins/Del in TP53, the frequencies of heterozygous TP5316-bp Ins/Del were 9.9% and 10.0% in patients and healthy controls (P = 0.94), respectively. The homozygote was not detected in either patients or controls. Thus, the TP5316-bp Ins/Del polymorphism was not included in further analysis. The distribution of the Arg/Arg, Arg/Pro and Pro/Pro genotypes of TP53 codon 72 Arg/Pro and their allele frequencies were not different between patients and controls (P > 0.05).
3.2 8-OHdG repair-related gene polymorphisms were associated with platinum sensitivity

The frequencies of the OGG1 c.977C > G, MTH1 c.247G > A, MUTYH c.972G > C, AluYb8MUTYH, TP53 codon 72 Arg/Pro and 16-bp Ins/Del genotypes and alleles were further compared between platinum-sensitive and refractory/platinum-resistant recurrent HG-SOCs, as shown in Table 4. Compared with platinum-sensitive recurrent HG-SOC, the distribution of the three genotypes for OGG1 c.977C > G, namely, CC, CG, and GG, and the allele frequencies were different to those of refractory/platinum-resistant recurrent cases ($P = 0.04$ and 0.03). The frequency of the OGG1 c.977GG genotype was statistically higher in the refractory/platinum-resistant recurrent cases (50.0%) than in the platinum-sensitive recurrent cases (35.4%), and the age-adjusted OR of GG was 1.83 (95% CI: 1.10–3.03; $P = 0.02$). For TP53 codon 72 Arg/Pro, the distribution of the three genotypes (Arg/Arg, Arg/Pro, and Pro/Pro) and the allele frequencies were different between refractory/platinum-resistant recurrent and platinum sensitive recurrent HG-SOCs ($P = 0.01$ and 0.05). The frequency of the TP53 codon 72 Arg/Arg genotype was statistically higher in the refractory/platinum-resistant recurrent cases (48.9%) than in the platinum sensitive recurrent cases (31.3%), with an age-adjusted OR for this genotype of 2.10 (95% CI: 1.26–3.51; $P = 0.01$). For MUTYH c.972G > C, the distribution of the three genotypes (GG, GC, and GG) and the allele frequencies were different in refractory/platinum-resistant recurrent cases and platinum-sensitive recurrent HG-SOCs ($P = 0.05$ and 0.04). The frequency of the MUTYH c.972 GG genotype was statistically higher in the refractory/platinum-resistant recurrent cases (42.2%) than in the platinum-sensitive recurrent cases (28.2%), with an age-adjusted OR for GG of 1.86 (95% CI: 1.10–3.13; $P = 0.02$).

3.3 8-OHdG repair-related gene polymorphisms influence 8-OHdG concentration

The 8-OHdG concentration in leukocyte DNA was evaluated in 100 randomly recruited HG-SOCs (cases) accord-
Table 3. The comparison on the frequencies of the variants detected between healthy controls and the HG-SOCs.

| Variation | Genotype | Healthy controls | HG-EOC patients | P-value* | OR (95% CI) |
|-----------|----------|------------------|-----------------|----------|-------------|
| hOGG1 c.977 C > G | C/C | 126 (18.0%) | 69 (19.7%) | 0.04 | |
|  | C/G | 366 (52.3%) | 155 (44.3%) | |
|  | G/G | 208 (29.7%) | 126 (36.0%) | |
|  | CC or CG | 492 (70.2%) | 224 (64.0%) | 0.04 | 1.33 (1.01–1.75) |
| C allele | 0.437 | 0.419 | |
| G allele | 0.563 | 0.581 | 0.19 | 1.26 (0.90–1.77) |
| MTH1 c.247 G > A | G/G | 646 (92.3%) | 329 (94.0%) | - | |
|  | G/A | 54 (7.7%) | 21 (6.0%) | |
|  | A/A | 0 | 0 | |
|  | GA or AA | 54 (7.7%) | 21 (6.0%) | 0.31 | 1.31 (0.78–2.21) |
| G allele | 0.961 | 0.97 | |
| A allele | 0.039 | 0.03 | 0.48 | 1.30 (0.63–2.68) |
| MUTYH c.972 G > C | C/C | 131 (18.7%) | 58 (16.6%) | 0.21 | |
|  | C/G | 362 (51.7%) | 170 (48.6%) | |
|  | G/G | 207 (29.6%) | 122 (34.8%) | |
|  | CC or CG | 493 (70.4%) | 208 (59.4%) | 0.02 | 1.40 (1.06–1.84) |
| C allele | 0.446 | 0.366 | |
| G allele | 0.554 | 0.634 | 0.16 | 1.16 (0.94–1.44) |
| AluYb8MUTYH | A/A | 230 (32.9%) | 105 (30.0%) | |
|  | A/P | 338 (48.3%) | 164 (48.7%) | |
|  | P/P | 132 (18.9%) | 81 (21.3%) | |
|  | A/P or P/P | 568 (81.1%) | 269 (78.7%) | 0.10 | 1.30 (0.95–1.77) |
| A allele | 0.570 | 0.534 | |
| P allele | 0.430 | 0.466 | 0.12 | 1.16 (0.96–1.39) |
| TP53 codon 72 Arg/Pro | Pro/Pro | 130 (18.6%) | 58 (16.6%) | 0.09 | |
|  | Arg/Pro | 342 (48.9%) | 154 (44.0%) | |
|  | Arg/Arg | 228 (32.6%) | 138 (39.4%) | |
|  | Pro/Pro or Arg/Pro | 472 (67.4%) | 212 (60.6%) | 0.03 | 1.35 (1.03–1.76) |
| Pro allele | 0.430 | 0.386 | |
| Arg allele | 0.570 | 0.614 | 0.17 | 1.20 (0.92–1.56) |
| TP53 16-bp Ins/Del | Del/Del | 631 (90.1%) | 315 (90.0%) | - | |
|  | Del/Ins | 69 (9.9%) | 35 (10.0%) | |
|  | Ins/Ins | 0 | 0 | |
|  | Del/Ins or Ins/Ins | 69 (9.9%) | 35 (10.0%) | 0.94 | 1.02 (0.66–1.56) |
| Del allele | 0.950 | 0.950 | |
| Ins allele | 0.050 | 0.050 | 0.96 | 1.02 (0.56–1.83) |

*P-value for comparison using χ² test to assess correlation between HG-SOC risk and predicted high-risk OGG1, MTH1, MUTYH, and TP53 genotypes and alleles.

* Genotypes were combined properly to assess their association with HG-SOC and the genotype 1.00 as the reference category.
OHdG concentration in subjects carrying the GG genotype was higher than in those carrying the CG or CC genotypes in patients with the TP53 codon 72 Pro/Pro (30.3 ± 5.7/10^6 dG vs. 23.2 ± 5.0/10^6 dG; P < 0.01). The median OHdG concentration in leukocyte DNA was distinguished as standard value. Based on these, the 100 patients were classified into two subgroups. Cox proportional hazards analysis revealed the OHdG concentration was independently associated with OS in HG-SOCs (P < 0.01, Table 5). The median OS and PFS durations of patients with higher concentration (30.2 and 12.6 months) were worse than those with lower concentration (40.4 and 22.0 months, P = 0.04 and < 0.01, respectively; Fig. 4).

4. Discussion

HG-SOC has the highest mortality of all epithelial ovarian cancers. The majority of HG-SOCs are initially sensitive to platinum but gradually develop resistance. The remaining patients are almost all platinum refractory ovarian cancer [20–23]. Most tumor cells can be destroyed by frontline chemotherapy. However, platinum resistant subclones with high adaptability to a new environment inevitably undergo relapse and drug resistance when selective pressure from platinum is applied. Genetic heterogeneity within cancers possibly facilitates drug-induced selection for an intrinsically resistant cancer subclone which then becomes dominant [20, 24–27].

TCGA analysis reveals that almost all HG-SOC harbor TP53 mutations that play an important early role in tumourgenesis. Most other mutations, including base excision repair-related gene mutations occur at low frequencies of approximately 5% of all cases [28]. Evidence shows that TP53 mutations act in coordination with OHdG repair-related gene inactivation in order to favor tumor progression [29, 30]. Although TP53 can regulate upstream of OHdG repair-related genes, the inactivated OHdG repair-related
Fig. 3. Combined analysis of the effect of polymorphisms in (b) OGG1 c.977C > G, MUTYH c.972G > C, and TP53 codon 72 Arg/Pro of HG-SOCs. Statistical significance was calculated by one-way ANOVA testing followed by post hoc analysis. *P < 0.05 and **P < 0.01 vs. subjects with the genotype in the blank bar.

Fig. 4. High 8-OHdG in leukocyte DNA is related with worse prognosis of HG-SOCs. (A) Higher 8-OHdG level was related with shorter OS (40.4 vs. 30.2 months, P = 0.04). (B) Higher 8-OHdG level was related with shorter PFS (22.0 vs. 12.6 months, P = 0.00).

genes provide feedback on the downregulation of TP53 under oxidative stress [31]. Accumulating evidence supports the hypothesis that polymorphisms in DNA repair-related genes modulate their ability to repair DNA damage, leading to an accumulation of DNA oxidative damage and genomic instability [32–36]. We previously reported that 8-OHdG repair-related gene polymorphisms associated with increased oxidative DNA damage could increase the risk of specific cancer types in a Chinese population (i.e., 8-OHdG) [9–11, 15, 16]. Another study revealed that increased 8-OHdG concentration in tumor DNA was correlated with poor OS and PFS in serous ovarian carcinoma [17]. Oxidative byproducts of cellular metabolism continuously generate oxidative DNA damage that can activate multiple repair
Table 4. The comparison on the frequencies of the variants detected between healthy controls and platinum sensitivity in HG-SOCs.

| Variation       | Genotype   | Healthy controls | Platinum-sensitive recurrent | Refractory/platinum-resistant recurrent | \( P \)-value\(^a\) | \( OR \) (95% CI) | \( P \)-value\(^b\) | \( OR \) (95% CI) | \( P \)-value\(^c\) | \( OR \) (95% CI) |
|-----------------|------------|------------------|-----------------------------|------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| hOGG1 c.977C>G  | C/C        | 126 (18.0%)      | 39 (20.0%)                  | 11 (12.2%)                               | 1.58           | 0.00           | 0.04           |                  |                 |                 |
|                 | C/G        | 366 (52.3%)      | 87 (44.6%)                  | 34 (37.8%)                               |                |                |                |                  |                 |                 |
|                 | G/G        | 208 (29.7%)      | 69 (35.4%)                  | 45 (50.0%)                               |                |                |                |                  |                 |                 |
| CC or CG\(^d\) | C allele   | 0.437            | 0.423                       | 0.311                                    |                |                |                |                  |                 |                 |
|                 | G allele   | 0.563            | 0.577                       | 0.689                                    | 0.52           | 0.93 (0.74–1.16) | 0.00           | 1.26 (1.75–2.44) | 0.03           | 1.53 (1.05–2.22) |
| MTH1 c.247G>A   | G/G        | 646 (92.3%)      | 183 (93.8%)                 | 81 (90.0%)                               |                |                |                |                  |                 |                 |
|                 | G/A        | 54 (7.7%)        | 12 (6.2%)                   | 9 (10.0%)                                |                |                |                |                  |                 |                 |
|                 | A/A        | 0                | 0                           | 0                                        |                |                |                |                  |                 |                 |
| GA or AA\(^d\) | G allele   | 0.961            | 0.969                       | 0.950                                    |                |                |                |                  |                 |                 |
|                 | A allele   | 0.039            | 0.031                       | 0.050                                    | 0.61           | 1.26 (0.51–3.11) | 0.61           | 1.31 (0.47–3.65) | 0.42           | 1.66 (0.48–5.78) |
| MUTYH c.972G>C  | C/C        | 131 (18.7%)      | 40 (20.5%)                  | 12 (13.3%)                               | 0.83           | 0.04           | 0.05           |                  |                 |                 |
|                 | C/G        | 362 (51.7%)      | 100 (51.3%)                 | 40 (44.4%)                               |                |                |                |                  |                 |                 |
|                 | G/G        | 207 (29.6%)      | 55 (28.2%)                  | 38 (42.2%)                               |                |                |                |                  |                 |                 |
| CC or CG\(^d\) | C allele   | 0.446            | 0.462                       | 0.356                                    |                |                |                |                  |                 |                 |
|                 | G allele   | 0.554            | 0.538                       | 0.644                                    | 0.32           | 0.85 (0.61–1.17) | 0.10           | 1.46 (0.92–2.30) | 0.04           | 1.71 (1.02–2.89) |
| MUTYH AluYb8   | A/A        | 230 (32.9%)      | 54 (27.7%)                  | 27 (30.0%)                               | 0.33           | 0.86           | 0.24           |                  |                 |                 |
|                 | A/P        | 338 (48.3%)      | 98 (50.3%)                  | 45 (50.0%)                               |                |                |                |                  |                 |                 |
|                 | P/P        | 132 (18.9%)      | 43 (22.1%)                  | 18 (20.0%)                               |                |                |                |                  |                 |                 |
| A/P or P/P\(^d\) | A allele  | 0.570            | 0.528                       | 0.550                                    | 0.32           | 1.22 (0.83–1.79) | 0.79           | 1.08 (0.62–1.86) | 0.69           | 1.13 (0.61–2.10) |
|                 | P allele   | 0.430            | 0.472                       | 0.450                                    | 0.51           | 1.19 (0.71–1.99) | 0.89           | 1.05 (0.46–2.28) | 0.63           | 1.09 (0.77–1.56) |
| TP53 codon 72 Arg/Pro | Pro/Pro | 130 (18.6%) | 36 (18.5%) | 10 (11.1%) | 0.93 | 0.01 |
|                 | Arg/Pro    | 342 (48.9%)      | 98 (50.3%)                  | 36 (40.0%)                               |                |                |                |                  |                 |                 |
|                 | Arg/Arg    | 228 (32.6%)      | 61 (31.3%)                  | 44 (48.9%)                               |                |                |                |                  |                 |                 |
| Pro/Pro or Arg/Pro\(^d\) | Pro allele | 0.430 | 0.436 | 0.324 |
|                 | Arg allele | 0.570 | 0.564 | 0.667 |
| TP53 16-bp Ins/Del | Del/Del | 631 (90.1%) | 175 (89.7%) | 79 (87.8%) | - |
|                 | Del/Ins    | 69 (9.9%)       | 20 (10.3%)                  | 11 (12.2%)                               |                |                |                |                  |                 |                 |
| Ins/Ins        | 0           | 0               | 0                           | 0                                        |                |                |                |                  |                 |                 |
| Del/Ins or Ins/Ins\(^d\) | Del allele | 69 (9.9%) | 20 (10.3%) | 11 (12.2%) |
|                 | Ins allele | 0.950 | 0.950 | 0.940 |

\(^a\)\(P\)-value for comparison using \(\chi^2\) test to assess correlation between platinum-sensitive recurrent HG-SOCs and controls in predicted high-risk OGG1, MTH1, MUTYH and TP53 genotypes and alleles.

\(^b\)\(P\)-value for comparison using \(\chi^2\) test to assess correlation between refractory/platinum-resistant recurrent HG-SOCs and controls in predicted high-risk OGG1, MTH1, MUTYH and TP53 genotypes and alleles.

\(^c\)\(P\)-value for comparison using \(\chi^2\) test to assess correlation between platinum-sensitive recurrent HG-SOCs and refractory/platinum-resistant recurrent ones in predicted high-risk OGG1, MTH1, MUTYH and TP53 genotypes and alleles.

\(^d\)genotypes were combined properly to assess their association with recurrent status and the genotype 1.00 as the reference category.
Table 5. The association of 8-OHdG concentration and overall survival in HG-SOC.

| Characteristic | Univariate analysis | Multivariate analysis |
|---------------|---------------------|-----------------------|
|               | HR                  | 95% CI                | HR                    | 95% CI               | P-value |
| Age           | 1.01                | 1.00–1.02             | 1.00                  | 0.98–1.02            | 0.43    |
| BMI           | 1.00                | 0.99–1.01             | 1.00                  | 0.99–1.00            | 0.76    |
| FIGO stage    | 1.44                | 1.10–3.58             | 1.15                  | 1.05–2.11            | 0.00    |
| I             | 1.00                | Reference             | 1.00                  | Reference            |         |
| II            | 1.55                | 0.64–6.82             | 1.20                  | 0.40–8.80            |         |
| III           | 5.29                | 1.62–16.25            | 2.12                  | 1.22–5.91            |         |
| IV            | 7.82                | 3.35–25.05            | 3.05                  | 1.40–8.42            | 0.00    |
| Neo-adjuvant chemotheraphy | 0.86 | 0.63–2.46 | 0.38 | 0.94 | 0.87–1.75 | 0.52 |
| Optimal CRS   | 0.65                | 0.44–0.86             | 0.72                  | 0.59–0.83            | 0.02    |
| Ascites       | 1.54                | 1.23–3.27             | 1.20                  | 1.06–2.30            | 0.04    |
| Nadir CA-125 level | 1.02 | 1.01–1.04 | 1.01 | 1.00–1.03 | 0.00 |

HR, hazard ratio; CI, confidence interval.

 pathways to maintain the genome. The 8-OHdG repair pathway plays a critical role in this repair. Several genotypes are associated with dysfunctional 8-OHdG repair and thus produce more drug resistant variations in DNA. These observations suggest that short-term recurrence and platinum resistant recurrence contribute to minor subpopulations of intrinsically resistant cancer cells at presentation. Combined genomic analysis of HG-SOCs by multiple temporally and spatially separate samples revealed deeply divergent mutational profiles and unique drug resistance evolutionary trajectories in all cases studied [37]. These results prompted us to explore the relationship between 8-OHdG repair-related gene polymorphisms and platinum sensitivity in HG-SOCs.

After confirming the relationship between the OGG1 c.977G > C variant and HG-SOC risk, we found that both the OGG1 c.977C > G and MUTYH c.972G > C polymorphisms were associated with sensitivity to platinum. OGG1 c.977GG and MUTYH c.972GG genotypes and allele frequencies were higher in refractory/platinum-resistant recurrent sub HG-SOCs than in platinum-sensitive recurrent HG-SOCs. The association between TP53 codon 72 Arg/Arg genotype and platinum-resistant HG-SOCs was consistent with previous results [38–40]. In the present HG-SOC population, OGG1 c.977C > G, MUTYH c.972G > C, and TP53 codon 72 Arg/Arg were significantly associated with the 8-OHdG concentration, both individually and in combination. It has been previously reported that OGG1 c.977CC has a 7-fold higher activity for 8-oxoguanine repair than OGG1 c.977GG [41]. Combined analysis of the synergistic effects of OGG1 c.977C > G, MUTYH c.972G > C, and TP53 codon 72 Arg/Pro polymorphisms revealed that MUTYH, OGG1, and TP53 may cooperate to prevent 8-OHdG repair.

The relapse time after first-line therapy indicates the platinum sensitivity in recurrent HG-SOC. Patients who have recurrence more than 6 months after the initial treatment are defined as having platinum sensitive ovarian cancer, while patients who recur within 6 months have platinum resistant ovarian cancer [42, 43]. Patterns of platinum response, relapse and the development of drug resistance are likely related to distinct molecular and cellular biological characteristics of HG-SOC [44, 45]. We first revealed that 8-OHdG concentration in leukocyte DNA was higher in refractory/platinum-resistant recurrent HG-SOCs than in platinum-sensitive recurrent sub HG-SOCs. Furthermore, Cox proportional hazards analyses revealed the 8-OHdG concentration was independently associated with the prognosis of HG-SOC. Moreover, higher 8-OHdG concentration was associated with poorer OS and PFS in HG-SOCs. These findings imply that 8-OHdG repair-related genes and high 8-OHdG concentrations in leukocyte DNA could eventually trigger more gene mutations and thus promote platinum-resistant variants. Platinum induces cross-linking within and between DNA strands. The subsequent single-strand and double-strand breaks cannot be directly repaired by 8-OHdG repair-related genes.

However, not all of the relationships observed in this study regarding 8-OHdG repair-related gene polymorphisms can be explained thoroughly. Some 8-OHdG repair-related gene polymorphisms were shown to be related to the inability to repair oxidative DNA, which can lead to the accumulation of 8-OHdG in leukocyte DNA. Therefore, we have found that a high level of 8-OHdG is associated with increased risk for HG-SOC. The associations between genetic variants, oxidative DNA damage, and disease state were not conclusive. For example, individual and combined analysis confirmed the MUTYH c.972G > C and TP53 codon 72 Arg/Pro variants significantly increased 8-OHdG levels, which predicted a high risk for oxidative DNA damage. These two variants were more frequent in refractory/platinum-resistant recurrent HG-SOCs, however they did not appear to be related to HG-SOCs risk in the Chinese population studied here. Carrying an inherited mutation in the BRCA1 or BRCA2 genes increases a woman’s lifetime risk of developing ovarian cancers, although there are considerable differences in disease manifestation [46]. Previous studies reported the rs2304277 variant in the OGG1 glycosidase gene of the Base Excision
Repair pathway can increase ovarian cancer risk [47, 48]. However, the relationship between 8-OHdG repair-related gene polymorphisms in our study and BRCA1/2 mutations has not been reported and needs further research.

In summary, our study using a Chinese cohort found that polymorphisms in the 8-OHdG repair system, including OGG1 c.977GG, increased the risk of HG-SOC. OGG1 c.977C > G, MUTYH c.972G > C, and TP53 codon 72 Arg/Pro polymorphisms were associated with sensitivity to platinum. The combination of MUTYH c.972GG, OGG1 c.977GG, and TP53 codon 72 Arg/Arg increased the 8-OHdG concentration in leukocyte DNA in HG-SOCs. An increased level of 8-OHdG was associated with poor prognosis of HG-SOC cases. Homozygous or heterozygous 8-OHdG repair-related gene polymorphisms might be candidate genetic factors for the development of platinum resistance. Screening for these polymorphisms and evaluation of the 8-OHdG concentration may help to prevent the progression of platinum resistance.

Abbreviations

8-OHdG, 8-Hydroxy-2'-deoxyguanosine; BER, base excision repair; HG-SOC, high-grade serous ovarian carcinoma.

Author contributions

JN participated in the design of present study and drafted the manuscript. YW and QZ performed the experiments. XX, XZC and HYG participated in the cases recruit of present study. WWG and RZ carried out statistical analysis. XXC designed of the study, WWG and RZcarried out statistical analysis. YL participated in the cases recruit of present study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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