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REVIEW

Pharmacogenomics of Cisplatin Sensitivity in Non-small Cell Lung Cancer

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Abstract Cisplatin, a platinum-based chemotherapeutic drug, has been used for over 30 years in a wide variety of cancers with varying degrees of success. In particular, cisplatin has been used to treat late stage non-small cell lung cancer (NSCLC) as the standard of care. However, therapeutic outcomes vary from patient to patient. Considerable efforts have been invested to identify biomarkers that can be used to predict cisplatin sensitivity in NSCLC. Here we reviewed current evidence for cisplatin sensitivity biomarkers in NSCLC. We focused on several key pathways, including nucleotide excision repair, drug transport and metabolism. Both expression and germline DNA variation were evaluated in these key pathways. Current evidence suggests that cisplatin-based treatment could be improved by the use of these biomarkers.

Introduction

Lung and bronchus cancer is the leading cause of cancer mortality worldwide, responsible for 1.59 million deaths in 2012 [1]. It is also the leading cause of cancer deaths in the US with a predicted 159,260 deaths in 2014 [2]. About 85% of all lung cancers are non-small cell lung cancer (NSCLC), whereas the remaining 15% are small cell lung cancer [3]. The standard of care for advanced (stages III and IV) NSCLC is cisplatin in combination with 1–3 of the following drugs including paclitaxel, gemcitabine, docetaxel, vinorelbine, irinotecan and pemetrexed, and concurrent radiotherapy for stage IIIB NSCLC patients [4]. Despite efforts to improve efficacy of therapy, the 5-year survival rate for NSCLC over all stages is still only 17% [5]. While many patients initially respond to cisplatin-based chemotherapy, others show intrinsic or acquired non-response [3]. Considerable efforts have been dedicated to the study of biomarkers for cisplatin sensitivity in NSCLC in order to improve therapeutic efficacy. In this review we will focus on three key pathways that are known to be associated with the effect of cisplatin on NSCLC. These three pathways/classes of enzymes are: nucleotide excision repair (NER), which represents drug targets; copper transporters, which play a role in drug uptake; and glutathione *S*-transferases (GSTs), which play a role in drug metabolism. For each of these pathways we reviewed current evidence for the use of mRNA expression and protein expression levels as biomarkers for cisplatin sensitivity. In addition, we discussed single

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nucleotide polymorphisms (SNPs) in these key genes and evidence for their involvement in cisplatin sensitivity in NSCLC.

General mechanism of action for cisplatin

Cisplatin is thought to enter the cell through a combination of passive uptake and active transport such as through the copper transporters [6]. Once in the cell, cisplatin becomes aquated and positively charged. It is then able to interact with and intercalate tumor DNA, forming cross-links and unwinding the helix; such DNA damage results in inhibition of DNA replication, eventually triggering apoptosis and necrosis (**Figure 1**) [7,8]. Platinating agents can form four types of links on DNA, *i.e.*, monoadducts (which typically become intrastrand crosslinks), intrastrand crosslinks, interstrand crosslinks and DNA-protein crosslinks [8]. In addition to DNA, cisplatin is able to react with thiol-containing compounds such as glutathione [8]. By forming glutathione adducts, cisplatin is detoxified, which reduces efficacy. After forming adducts with DNA, the cisplatin molecules activate the endoplasmic reticulum (ER) stress pathway (also known as the unfolded protein response), thereby activating apoptotic caspases [8]. Sensitivity to cisplatin is known to be mediated by multiple different mechanisms likely working synergistically to prevent, detect and remove DNA adducts formed by cisplatin. In this review we focused on NER, copper transport and GSTs as proxies for drug action, uptake and metabolism, respectively (Figure 1). Our goal is to present a broad picture of cisplatin's mechanism of action while simultaneously examining each of these pathways in detail for their role in cisplatin sensitivity.

NER and cisplatin sensitivity

Over 90% of DNA adducts formed by cisplatin are 1,2-intrastrand d(GpG) [9] or 1,2-intrastrand d(ApG) crosslinks [9,10].

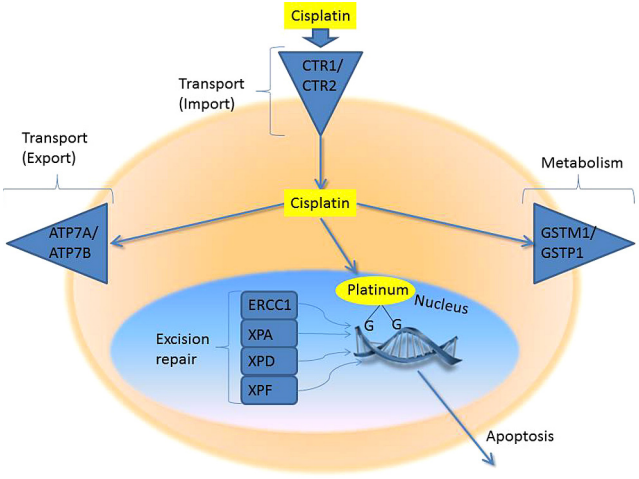

Figure 1 Schematic diagram of cisplatin's mechanism of action
The three representative pathways discussed in the text are illustrated, including drug action, uptake and metabolism. ATP7A and ATP7B, ATPase, copper transporting, alpha polypeptides A and B; CTR1 and CTR2, copper transporters 1 and 2; ERCC1, excision repair cross-complementation group 1; GSTM1 and P1, glutathione *S*-transferases Mu 1 and pi 1; XPA, XPD and XDF, xeroderma pigmentosum complementation groups A, D and F.

Such adducts, in addition to other less common crosslinks, are known to be detected and excised by NER [11]. Further, strong evidence for significant pharmacogenomic associations exists for genes involved in the NER pathway, indicating treatment could be improved by clinical assessments of the genes in the NER pathway.

NER mechanism of action

The exact mechanism of action underlying the role of NER in sensitivity to cisplatin has been discussed at length elsewhere [12,13] and is not reviewed in detail in the present article. In brief, NER is responsible for the detection and removal of bulky DNA adducts by means of ssDNA excision. There exist two distinct modes for the detection of DNA damage within NER—global genomic NER (GG-NER) and transcription coupled NER (TC-NER). These two modes are identical in essence except for damage detection. While GG-NER requires the use of xeroderma pigmentosum complementation group C (XPC) and deafness/dystonia proteins (DDP) to actively detect damage, TC-NER is passively activated when RNA polymerase stalls at the damage site. Furuta and colleagues found that cisplatin sensitivity is mediated through TC-NER exclusively [14], while Rosell et al. reported that there is evidence for both modes of NER modulating cisplatin resistance [15]. After damage detection, excision repair cross-complementation group 1 (ERCC1) forms a complex with xeroderma pigmentosum complementation group F (XPF) and creates an incision ~23 nucleotides upstream from the DNA adduct. Cell lines defective in *ERCC1* or *XPF* are approximately 40 times more sensitive to cisplatin [16], indicating that this step is critical in cisplatin sensitivity.

ERCC1 and cisplatin sensitivity in NSCLC

ERCC1 is the rate-limiting protein in the NER pathway and has therefore been a subject of many studies attempting to explain cisplatin resistance. The first report on ERCC1's role in cisplatin resistance came from Dabholkar and colleagues in cells harvested from ovarian cancer tissue. Tissues from patients whose tumors showed clinical resistance to cisplatin exhibited significantly higher levels of *ERCC1* mRNA ($P = 0.026$) [17]. Since then, there have been a variety of reports indicating that *ERCC1* mRNA and protein expression levels in tumors are indicative of cisplatin sensitivity in NSCLC. Some researchers have measured cisplatin sensitivity directly by means of cell viability tests after treatment. Takenaka and colleagues reported that high protein expression level of *ERCC1* is significantly correlated with low cisplatin chemosensitivity ($P = 0.04$) using tumor biopsies collected from 41 NSCLC patients before treatment [18]. Wang and colleagues used an ATP-based tumor chemosensitivity array (ATP-TCA) to assess cell viability and found that increasing levels of *ERCC1* mRNA expression were correlated negatively with cisplatin sensitivity in primary tumor cells collected from 20 NSCLC patients ($P = 0.001$) [19]. A similar study, using the WHO tumor response guidelines and a combination cisplatin/gemcitabine treatment, found that while there is a significant correlation between higher response rates and low expression levels of *ERCC1* mRNA in pretreatment primary NSCLC tumor specimens, there was no significant difference in *ERCC1* mRNA levels detected between responding and non-responding tumors after the treatment [20]. The authors

of this study hypothesized that the lack of significant correlation of post-treatment *ERCC1* expression with overall response is not contradictory to the above studies; rather, the pretreatment levels of *ERCC1* expression are a good indicator for cisplatin chemosensitivity. This hypothesis was tested in a phase III trial of 346 NSCLC patients, where treatment was customized based on the pretreatment levels of *ERCC1* mRNA expression in the patients. Specifically, patients with high level *ERCC1* expression were treated with docetaxel plus gemcitabine, whereas patients with low *ERCC1* expression received docetaxel plus cisplatin. The significantly higher response rates were observed in the docetaxel plus cisplatin group ($P = 0.02$) [21]. Other studies also corroborated the link between *ERCC1* expression levels and sensitivity to cisplatin in NSCLC patients including a study of the link between *ERCC1* expression levels in 761 NSCLC tumors and efficacy (prolonged survival) of cisplatin-based adjuvant chemotherapy ($P = 0.009$) [22] (**Table 1**).

However, ERCC1 is not a perfect biomarker for cisplatin sensitivity, as a number of studies found a lack of correlation between the two. Wachters and colleagues reported no significant correlation between *ERCC1* expression levels and tumor response in group of 33 stage III/IV NSCLC patients [46]. Similarly, Booton et al. found no significant relationship between *ERCC1* mRNA expression and cisplatin sensitivity in a group of 66 stage III/IV NSCLC patients [47]. One explanation for the lack of significant correlation is that these studies are based on patients treated with combination therapy including cisplatin. The addition of other drugs, such as gemcitabine or docetaxel, on tumor response can be a confounding factor. Another study by Glaysher and colleagues used an ATP-TCA to assess chemosensitivity in 49 surgically-resected NSCLC tumor samples and fit multiple-regression models to explain sensitivity based on multiple gene expression profiles. Their model did not find *ERCC1* expression to be a significant explanatory variable for cisplatin resistance compared to the other genes present [48]. This result is surprising considering the evidence above, but given the method that the models in that study were constructed with, it is possible that some combination of other predictive genes do better together than *ERCC1* expression does alone. A few other studies which failed to find a correlation between *ERCC1* expression and cisplatin sensitivity suffer from similar drawbacks (*i.e.*, confounds from additional drugs other than cisplatin, models not specific to *ERCC1* expression, or inconsistency in *ERCC1* expression quantification methods used [49,50]) and do not constitute sufficient evidence to rebut those studies that do find a significant link. Considering the large amount of evidence cited above in contrast to the limited contradictory evidence, we conclude that *ERCC1* expression is a potentially reliable biomarker for cisplatin sensitivity in NSCLC patients when reliable detection techniques are available. This conclusion is supported by a comprehensive and comparative review, which considered many different factors (including the biomarkers discussed in this review) and concluded that the strongest evidence for increased resistance in NSCLC to platinating agents is the expression of *ERCC1* [51].

With this connection in mind, there have been many studies attempting to link SNPs in *ERCC1* with cisplatin sensitivity; however, findings are often contradictory. SZ Wei et al. performed a meta-analysis of 12 datasets from 556 NSCLC patients consisting of genotypes and clinical outcomes to cisplatin treatment and found a significant correlation between overall response rate (ORR) and the rs11615 polymorphism (**Table 2**) [52]. Similarly, HB Wei et al. performed a meta-analysis on 11 datasets and confirmed the findings of SZ Wei and colleagues [53]. However, Yu et al. performed a similar meta-analysis of 1252 NSCLC patients and concluded there was no significant link between this polymorphism and response to cisplatin treatment [54]. They claimed that their study more accurately represented the underlying data and that it had greater statistical power due to larger sample size. Possible explanations for these discrepancies include confounders like race, age, smoker status. Clearly there is a need for a more comprehensive study of *ERCC1* polymorphisms in relation to cisplatin response.

XP family genes and cisplatin sensitivity in NSCLC

Also involved in the NER pathway is the family of *XP* genes and their encoded proteins. Although it is well recognized that

Table 1 Gene expression correlation with cisplatin sensitivity in NSCLC

Gene name	Correlation between expression and sensitivity	Evidence	Refs.
ERCC1	↓	Clinical study conducted in 761 patients	[21,22]
XPA	↓	Human lung cancer cell lines	[23–25]
CTR1	↑	Clinical study conducted in 54 patients	[26]
	↑	Human small cell lung cancer cell lines	[27]
	↑	Yeast and mouse cell lines	[28,29]
ATP7A	↓	Clinical study conducted in 89 patients	[30]
	↓	Human NSCLC cell lines	[30,31]
ATP7B	↓	Clinical study conducted in 104 patients	[32]
	↓	60 human tumor cell lines including NSCLC	[33,34]
	↓	NSCLC xenografts	[35]
GSTP1	↓	Cell lines, tumor samples and clinical study tracking expression and survival	[36–42]
STM1	↔	Animal cell lines and human breast cancer cell lines	[43,44]
	↓	Human lung cancer cell line	[45]

Note: Increase and decrease in cisplatin sensitivity in response to increased gene expression are indicated with ↑ and ↓, respectively; whereas ↔ indicates that cisplatin sensitivity did not change regardless of alterations in gene expression.

Table 2 Evidence for correlation of germline genetic variants with cisplatin sensitivity in NSCLC

Variant ID	Host gene	Effect	Evidence	Refs.
rs11615	*ERCC1*	T/T genotype associated with low sensitivity	Conflicting meta-analyses of clinical studies	[52–54]
rs13181	*XPD*	A/C and C/C genotypes associated with high sensitivity in Caucasian populations and low sensitivity in Asian populations	Meta-analysis of clinical studies	[55]
rs7851395	*CTR1*	Associated with low sensitivity in Asian population	Clinical study conducted in 282 patients	[56]
rs12686377	*CTR1*	Associated with low sensitivity in Asian population	Clinical study conducted in 282 patients	[56]
rs1695	*GSTP1*	G allele associated with favorable response; A/G + G/G genotypes more likely to be responders	Conflicting evidence: various clinical studies and one meta-analysis	[3,57–61]
rs1138272	*GSTP1*	Ala/Val or Val/Val genotype associated with greater median survival	Clinical studies	[3,61]
GSTM1*0	*GSTM1*	Null genotype associated with increased sensitivity compared to non-null	Meta-analysis of clinical studies	[62]
rs560018	*GSTM4*	A/G genotype associated with decreased survival G/G genotype associated with decreased sensitivity	Clinical study in 973 lung cancer patients 100 lymphoblastoid cell lines	[63] [63]

the XP family of genes play a critical role in NER, there is little evidence that expression levels of most XP- proteins are correlated with sensitivity to cisplatin. Perhaps the best evidence is in *XPA*. *XPA* is known to be involved in both DNA damage detection in GG-NER and recruiting NER repair proteins (*e.g.*, ERCC1/XPF complex) to the site of damage. Wu and colleagues found in two separate studies that by transfecting a human lung cancer cell line with antisense *XPA* RNA, mRNA levels of *XPA* are reduced and sensitivity to cisplatin as measured by an MTT metabolic assay is increased [64,23]. Similarly, Zhang and colleagues showed that by silencing *XPA* expression in a cisplatin-resistant NSCLC cell line, sensitivity to cisplatin is increased [24]. Another study found that by modulating HIF1α, a transcription factor known to bind to the *XPA* promoter, *XPA* expression levels can be increased or decreased, thereby decreasing or increasing cisplatin resistance, respectively [25]. Finally, Rosenberg and colleagues found that by transfecting a human lung cancer line with a virus expressing a truncated version of the XPA protein, sensitivity to cisplatin can be increased [65]. Although these findings in cell lines warrant further investigation, the lack of clinical evidence to date decreases the level of enthusiasm in using the XP family proteins as markers for cisplatin sensitivity (Table 1).

Certain SNPs in *XP* family genes have shown promise in cisplatin sensitivity prediction. Specifically, rs13181 located in the coding region for *XPD* has been intensively studied for its effect on both lung cancer risk and cisplatin sensitivity. Qin et al. performed a meta-analysis of 24 studies on platinum-based chemotherapy for NSCLC patients and found that when the analysis was stratified by race, the A/C and C/C genotypes were significantly associated with favorable objective response in Caucasian populations, but among Asian populations they found an association with the same polymorphisms and decreased progression-free survival [55] (Table 2). SNPs in other *XP* family genes have garnered some preliminary evidence for their use as biomarkers of cisplatin sensitivity, but comprehensive meta-analysis or large-cohort studies have not been done in order to achieve sufficient statistical power.

The lack of correspondence between mRNA/protein expression studies and genetic variation studies along with the multiple contradictory findings in sufficiently-powered analyses indicates that the relationship between genotype and response may not be a simple one-to-one function. A more nuanced approach which looks at multiple SNPs within the NER pathway in conjunction has been shown to be a potentially-powerful tool in predicting cisplatin sensitivity [57].

Copper transporters and cisplatin sensitivity

As discussed above, sensitivity to cisplatin is multifactorial and the relative contribution of different pathways is still not well elucidated. However, it has long been noted that cisplatin-resistant cells tend to exhibit decreased intracellular drug accumulation [66]. A recent study has established a significant correlation between intracellular platinum buildup and reduction in tumor size [67]. It was previously thought that the dominant mode for cisplatin's entry into tumor cells was through passive diffusion [66], but growing evidence has supported the role of copper transporters in the active influx and efflux of cisplatin [67].

Mechanism of action of copper transporters

Copper serves an important role in biological processes as a cofactor for many enzymes. The intake of copper is thought to be mediated by the protein human copper transporter 1 (hCTR1) encoded by the *CTR1* (or *SLC31A*) gene [68]. The copper is then chaperoned by ATX1, CCS and COX17 to ATPase, copper transporting, alpha polypeptide A and B (ATP7A and ATP7B), Copper-zinc superoxide dismutase and cytochrome C oxidase, respectively [69]. Excessive intracellular copper has been found to be regulated by two proteins, ATP7A [70] and ATP7B [71]. While the role of this pathway has been extensively studied with regard to copper transportation, only in the past decade and a half has attention been paid to the role it might play in cisplatin transport and sensitivity. Safaei and Howell [69] reviewed evidence based on cross-resistance between copper and platinum drugs to demonstrate that CTR1 and ATP7A/ATP7B play an important role in the active regulation of intracellular Pt agents and corresponding sensitivity. Kuo et al. additionally reviewed the structural-functional and mechanistic aspects of these transporters [6].

CTR1/CTR2 and cisplatin sensitivity

The main body of work on the effects of copper transporters on cisplatin sensitivity has been done mostly in cancers other than NSCLC. However, it is becoming clear that these effects

are analogous across different cancer types. We therefore include evidence in cancers other than NSCLC, as one can likely extrapolate between the various diseases. *CTR1* was first shown to be related to cisplatin uptake and sensitivity in strains of the yeast *Saccharomyces cerevisiae* in two studies. *CTR1* knockout yeast strains were shown to have a twofold reduction in cisplatin uptake and cytotoxic effect of cisplatin compared to wild-type *CTR1* strains [28]. These results were confirmed in yeast and extended to mouse cell lines [29]. Considering the highly-conserved nature of the copper transport pathway, these findings lead researchers to investigate the effects of *CTR1* in humans. Song et al. demonstrated that at least one strain of a cisplatin-resistant small cell lung cancer (SCLC) cell line exhibited reduced levels of *CTR1* mRNA compared to a cisplatin-sensitive cell line. They further showed that *CTR1*-transfected SCLC cells had an increase in cisplatin sensitivity [27]. Holzer et al. showed that in murine embryonic fibroblast cell lines, *CTR1* deficiency was associated with decreased accumulation of and increased resistance to cisplatin [72]. Zisowsky et al. similarly showed that cisplatin-resistant cervical and ovarian cancer cell lines exhibited reduced levels of cisplatin accumulation and 1.5−1.8 fold reduction in *CTR1* mRNA expression [73]. These experimental results have been reproduced in a number of subsequent studies [74–76]. Ishida et al. provided one of the first studies of the clinical importance of *CTR1* mRNA expression. They found in two separate studies involving 15 ($P = 0.016$) and 91 ($P = 0.003$) patients that high expression of *CTR1* mRNA was correlated with increased disease-free survival in advanced ovarian cancer when treated with platin-based therapy [77].

Chen et al. extended these results to NSCLC and found that in pre-treatment samples from 54 advanced NSCLC patients treated with cisplatin or carboplatin, high *CTR1*protein expression level was a significant prognostic factor for favorable chemotherapy response, progression-free survival ($P = 0.01$) and overall survival ($P = 0.047$) [26]. In an intriguing pilot study, Fu et al. found that by pretreating patients with a copper-lowering agent in order to re-sensitize platinum-resistant ovarian cancer tumors, four out of five patients exhibited increased response to carboplatin. The proposed method of action of this copper-lowering agent is that high levels of copper cause a down-regulation of *CTR1* and that by removing some of the extracellular copper, *CTR1* will be up-regulated and allow for greater influx of carboplatin into tumor cells [78].

These results are promising in terms of using *CTR1* as a prognostic indicator for cisplatin sensitivity in NSCLC. However, more clinical evidence is needed before any conclusions can be drawn. Ivy et al. recently suggested that although *CTR1* expression is correlated with platinum uptake, the major entry pathway of platinum-based agents cannot be saturated by excessive platinum [79]. Hall et al. reviewed effects of cellular accumulation of platinum-based drugs on sensitivity and argued that the ability of CTR1 to transport platinating agents is tissue-specific and that there is a possibility that the platinum transported by CTR1 is not cytotoxic [80]. Overall, studies about the impact of CTR1 on cisplatin sensitivity are largely lacking in NSCLC. While we may be able to extrapolate from studies in other cancers, their use is limited. Clearly more studies are needed to elucidate the potential role of *CTR1* as a biomarker or target for treatment, but there is compelling evidence that warrants larger and more powerful studies.

CTR2 is located close to *CTR1* on the human chromosome arm 9q32; however, their respective mRNAs exhibit 0% homology and their proteins share 33% protein identity [81]. Less intensively-studied than *CTR1*, *CTR2* has recently been shown to also have an impact on cisplatin sensitivity, albeit in the opposite manner of *CTR1*. Blair et al. found that knockdown of *CTR2* increased uptake of cisplatin and cytotoxicity in cell lines of mouse embryo fibroblasts. They further found a significant correlation between *CTR2* mRNA and protein levels and cisplatin sensitivity in six ovarian cancer cell lines [82]. Further studies in cancer cell lines [83] and some preliminary clinical studies (reviewed by Ohrvik et al.) [84] provided some evidence for the role of CTR2 in cisplatin sensitivity. However, to date there are no studies of *CTR2* in NSCLC. Therefore we cannot draw conclusions regarding the impact of CTR2 on cisplatin sensitivity in NSCLC at the current time.

ATP7A/ATP7B and cisplatin sensitivity

As mentioned above, both ATP7A and ATP7B are involved in the efflux of copper from mammalian cells. In contrast to *CTR1*, which is expressed in nearly every tissue in the body, *ATP7B* is mainly expressed in the liver, kidney and brain, while *ATP7A* is expressed in most tissues aside from the liver [69]. Mutations in *ATP7A* and *ATP7B* are the causes of Menkes [70] and Wilson's disease [71], respectively. Deficiency in copper efflux is the main cause of excessive copper accumulation in these two diseases, which leads to the hypothesis that these efflux genes may be involved in the efflux of cisplatin as well. Samimi and colleagues found that an increase in *ATP7A* expression in post-treatment tumor samples is associated with poor survival in ovarian cancer patients ($P = 0.0057$) [85]. In another study, they found that increased expression of *ATP7A* is correlated with resistance to cisplatin in ovarian cancer cell lines, but not with decreased accumulation of intracellular platinum [86].

Similar results have been observed in NSCLC. Li et al. found that a cisplatin-resistant NSCLC cell line that expressed significantly higher levels of *ATP7A* mRNA and protein than the sensitive parental cell line can be partially re-sensitized by silencing *ATP7A* with siRNA. They further found that the presence of *ATP7A* in pretreatment tumor samples from 89 NSCLC patients was associated with worse response to cisplatin-based chemotherapy, compared to patients negative for *ATP7A* ($P = 0.001$) [30]. Recently Song et al. identified miR-495, an miRNA that directly targets *ATP7A*. They found that overexpression of miR-495 negatively regulates *ATP7A* and can partially restore cisplatin sensitivity in a cisplatin-resistant NSCLC cell line [31]. This finding provides an intriguing possibility for a cisplatin-sensitivity biomarker, but conclusive evidence is currently lacking.

ATP7B was first proposed to be related to cisplatin sensitivity by Komatsu et al. when they found that human epidermoid carcinoma cell lines transfected with *ATP7B* exhibited a 9-fold increase in resistance to cisplatin and a decrease in cisplatin accumulation [87]. Nakayama et al. found that the presence of *ATP7B* mRNA and protein in samples collected from 104 ovarian cancer patients was a significant prognostic factor for poor response to cisplatin-based chemotherapy ($P = 0.048$) [32]. In a broad study of 60 cancer cell lines, Konkimalla et al. assessed the levels of 55 candidate

transporter genes for association with cisplatin resistance. They found that among 17 genes exhibiting significant correlation (including *CTR1*), *ATP7B* had the lowest *P* value at 0.0006 [33]. Inoue and colleagues were the first to establish the positive correlation between *ATP7B* mRNA levels and cisplatin resistance in NSCLC tumor samples ($P = 0.015$) [34]. Recently the same correlation was demonstrated *in vivo* by correlating *ATP7B* mRNA ($P = 0.0389$) and protein ($P = 0.0357$) expression levels with cisplatin sensitivity in NSCLC xenografts [35]. These findings are promising but do not constitute sufficient evidence to conclude that *ATP7B* is a reliable biomarker for cisplatin sensitivity in NSCLC.

At the present time, research into potential germline polymorphisms in copper transport genes as biomarkers for cisplatin sensitivity is limited by the lack of potential candidate SNPs in those genes [88]. A recent meta-analysis of pharmacogenomics examined the use of platinum-based therapy for the treatment of NSCLC, no genes involved in the copper transport pathway were examined [3]. There is one study on the effects of SNPs in *CTR1* on cisplatin sensitivity. Xu et al. found that in 282 Chinese Han NSCLC patients treated with platinum-based chemotherapy, two SNPs, rs7851395 and rs12686377, in *CTR1* were correlated with both platinum resistance and overall survival ($P < 0.05$, Table 2) [56]. No studies were reported that relate SNPs in *ATP7A*/*ATP7B* to cisplatin sensitivity. However, in one study involving 203 Japanese cancer patients, Fukushima-Uesaka et al. identified 38 and 61 genetic variations in *ATP7A* and *ATP7B*, respectively [89]. While this study does not provide us with any insights into cisplatin treatment in NSCLC, it provides researchers with some potential candidate SNPs to evaluate.

GST and cisplatin sensitivity

Finally, we review the role of GSTs, a class of phase II drug metabolizing enzymes, in the efficacy of cisplatin. GSTs are involved in detoxification of cisplatin and aid in the formation of (inactive) platinum-glutathione conjugates, which can be excreted or further metabolized due to their now-increased solubility (see Figure 1) [58,88]. The rationale behind investigating GSTs is that patients with null or deficient GST function may experience decreased platin detoxification and an increased sensitivity to cisplatin, possibly as a result of increased active cisplatin concentrations. The focus in the literature has been primarily on GST pi 1 (GSTP1) because it is expressed in different human epithelial tissues and is "the most abundant GST isoform in the lung" [58,90,91]. *GSTM* genes have also been widely investigated, in part due to their highly polymorphic nature (see section below). Here we discuss the expression and genetic polymorphisms of genes in the *GSTP* and *GSTM* families and their associations with cisplatin sensitivity.

GST mechanism of action

The mechanism of action of GSTs is relatively simple. Aquated cisplatin can bind to DNA to form adducts; however aquated cisplatin can also be inactivated via conjugation with glutathione [88]. Glutathione is conjugated to substrates, such as platinum, through the action of GSTs. These glutathione-platinum conjugates have increased solubility and can be excreted by the ATP-binding cassette, subfamily C (ABCC) transporter family [63,88].

GSTP1 and cisplatin sensitivity

Previous research on GSTP1 focused on distinguishing human lung cancer cell lines. In 1988, using Northern blot hybridization analysis, Nakagawa et al. found that the levels of *GSTP1* mRNA were significantly higher in 3 NSCLC cell lines, compared to SCLC cell lines [36]. Using a colony forming assay to establish the IC50s for cisplatin for various cell lines, they found that the IC_{50} of the SCLC were 3–6.67 times lower than those for NSCLC cell lines. High sensitivity in SCLC cell lines was correlated with low levels of *GSTP1* mRNA expression [36]. These results suggested that low levels of *GSTP1* might be responsible for higher cisplatin sensitivity. In 1997, Arai et al. showed that patients with *GSTP1*-negative tumors had significantly better response than patients with *GSTP1*-positive tumors [37]. Their study involved 60 patients (49 men and 11 women) who had previously untreated and unresected NSCLC. These patients received cisplatin and etoposide and tumor response was assessed when maximum tumor reduction was clinically observed. 66.7% (16/24) of patients with *GSTP1*-negative tumors were partially responsive, compared to 25% (9/36) of patients with *GSTP1*-positive tumors. They concluded that there is a significant correlation between the chemotherapy response and *GSTP1* expression (characterized by immunohistochemical staining and validated by RT-PCR) ($P = 0.0019$) [37]. A similar study by the same group replicated their initial findings: Patients with negative *GSTP1* expression were more likely to be partial responders or have a higher response rate than patients with positive *GSTP1* expression (*GSTP1*-positive tumors) [38]. It is worth noting that both studies had recruited more males than females, whereas gender could be a potential confounding variable in response.

To validate and further elucidate this relationship, Hida et al. examined 11 NSCLC cell lines established from patients who had not previously undergone treatment and observed increased *GSTP1* expression in cell lines with a higher IC_{50} for cisplatin-based chemotherapy [39]. However, this association disappeared for cell lines derived from patients who had previously received some combination chemotherapy (including cisplatin-based chemotherapy). Furthermore, cell lines established from patients before and after chemotherapy showed no increase in the amount of GSTP1 protein. Cell lines with acquired resistance to cisplatin also showed no increase in the amount of GSTP1 protein. Taken together, these results confirm the observation of Arai and colleagues, but also imply that *GSTP1* may play a role in innate, but not acquired resistance of tumors.

Hirano et al. analyzed *GSTP1* levels from 126 surgically-resected NSCLC tumors as well as in two normal and cisplatin-resistant cultured lung cancer cell lines, H69 and PC14 [40]. In the H69 cells, they observed an increase in *GSTP1* expression in the resistant strain compared to the parent strain. In addition, they found *GSTP1*-negative patients showed significantly higher survival than *GSTP1*-positive patients when treated with platinum-based adjuvant therapy ($P = 0.0219$). This is only true in those NSCLC patients

who received postoperative adjuvant therapy—as opposed to patients who received no treatment after resection, indicating that *GSTP1* expression is associated with survival only in patients treated with cisplatin.

Similarly, Allen et al. found that higher *GSTP1* nuclear staining was significantly associated with decreased survival in stage I and II squamous cell carcinomas, a subtype of NSCLC ($n = 40$, $P = 0.02$) [41]. Cytoplasmic staining showed a similar trend, although it did not reach statistical significance ($P = 0.15$). This is not surprising since the proposed mechanism of action for GSTs is in the nucleus. In contrast to Hirano et al.'s work, they found no significant correlation between *GSTP1* staining and survival for other histological types of NSCLC. The discrepancy could be explained by the failure of Hirano et al. to differentiate between histopathological cell types in their analysis.

Rosell et al. analyzed expression of *GSTP1* in mRNA isolated from pretreatment biopsies of patients with metastatic NSCLC [42]. In contrast to the previous studies, there were no differences in response observed by treatment arms when broken down by *GSTP1* expression. The authors also found that *GSTP1* was not associated with time to progression or survival. The lack of association could be explained by the low *GSTP1* expression in late stage metastatic lung cancer. It would be consistent with Allen and colleagues' findings that *GSTP1* expression is significant only in stages I and II for squamous cell carcinoma. Interestingly, it has been shown that inhibition of nuclear transport of GSTP results in increased sensitivity to cisplatin in colon, glioblastoma and lung adenocarcinoma cell lines (a histopathological subtype of NSCLC) [41].

These results are promising; however, based on low cisplatin-glutathione conjugation rates, a recent study by Peklak-Scott et al. concluded that cisplatin resistance attributed to *GSTP1* is not caused by cisplatin conjugation, but rather modulation of other signaling pathways [92]. In a review of glutathione-related enzymes, Tew suggests that the association between *GSTP1* expression and cisplatin sensitivity is likely a by-product of pleiotropic stress as opposed to an active mechanism [93]. The precise nature of the relationship between *GSTP1* expression and cisplatin sensitivity is still unclear, but their association has been observed consistently and may serve as a useful biomarker.

Genetic polymorphisms in *GSTP1* have been studied to greater extent than its expression both for susceptibility to various cancers and for sensitivity to anticancer drugs. Here we discuss two such polymorphisms: rs1695 and rs1138272 (Table 2). Among the studies reviewed below, there are disparate results. Some studies confirmed a general trend for the association of rs1695 with decreased cisplatin sensitivity, while a number of other studies found that this SNP is associated with favorable response and increased survival. Yet other studies found that the association is insignificant. There is evidence that the rs1138272 is associated with prolonged survival.

Polymorphism rs1695 is a substitution of a wild type A allele to a G allele, which results in an amino acid substitution of a valine to an isoleucine. Such substitution affected the conjugate ability of GSTP1 to various substrates including platinating agents [94,95] and decreased GSTP1 activity in a gene/dose-dependent manner [94].

Sun et al. analyzed 113 Chinese Han patients that were diagnosed with stage IIIA-IV histologically-confirmed NSCLC and were on one of three platinum-based chemotherapies at 4 loci using a gel-based DNA microarray genotyping method [58]. They conclude that the presence of rs1695 variant differed significantly between responders and non-responders. After adjusted for various factors (gender, age at diagnosis, chemotherapy regimens), the odds ratio for response was 2.881 ($P = 0.022$) for those carrying at least one variant allele. In other words, patients carrying the G variant allele at this locus were more likely to be cisplatin responders. Similar observations were also reported in a recent study by Zhou and colleagues. They examined two SNPs including rs1695 using blood samples from 111 stage IV Chinese NSCLC patients treated with platinum-based therapy [59]. The authors found that rs1695 variant allele was associated with favorable response to platinum-based chemotherapy (adjusted $P = 0.0005$). A meta-analysis of 24 publications performed independently by 2 groups on 11 polymorphisms in 8 genes found that rs1695 was significantly associated with favorable response to platinum-based chemotherapy, although the effect was moderate and was only observed in Asian populations ($P = 0.0002$) [3]. However, it is worth nothing that the sub group that confirmed this consisted only of two publications [58,59].

However, other studies found opposite direction SNP association. Wu et al. analyzed 13 SNPs in cisplatin-related pathways, including rs1695, in 229 patients with stage IIIB or IV NSCLC receiving first-line cisplatin-based chemotherapy. The variant allele of rs1695 was significantly associated with decreased survival (hazard ratio (HR) = 1.99, $P = 0.015$); however, such association was not significant after adjusting for multiple comparisons [57]. Similarly, Booton et al. analyzed 4 known polymorphisms, including rs1695 and rs1138272, in exons 5 and 6 of *GSTP1* in 108 patients, and found each addition of a *B allele, defined by the authors as having both one variant rs1695 and one variant 1138272 allele, resulted in a non-significant inferior tumor response and survival, quantified by a 22% increased risk of death, compared to those who had wild type alleles of rs1695 and rs1138272 [60]. Furthermore, patients with variant rs1138272 demonstrated greater median survival and a reduction in HR of death of 0.56, which has not been addressed by other authors. It is worth noting that one of the treatment arms involves carboplatin and docetaxel (as opposed to cisplatin, mitomycin and ifosfamide or cisplatin, mitomycin and vinblastine); however, despite the use of carboplatin in one of the treatment arms, they found no significant difference between treatment arms [60]. The lack of significance of the *B allele may be a result being underpowered. In combination with the study by Wu and colleagues, these results warrant further investigation of rs1695.

Lu et al. genotyped 424 and 425 stage III or IV NSCLC patients treated with platinum-based chemotherapy for *GSTP1* genotypes in exon 5 and exon 6, respectively [61]. In contrast to all aforementioned studies, they found that *GSTP1* SNPs were not associated with survival. However, multivariate analysis revealed a positive association between rs1138272 variant and prolonged survival compared to wild type with an adjusted HR of 0.75. This effect was only observed for male patients younger than 62. The authors stated that the lack of follow-up clinical data and treatment information prevent them from concluding that rs1138272 is correlated with cisplatin response. Of note, the authors did not specify any drug

regimen for the patients, but simply asserted that these patients were likely on some form of platinum-based chemotherapy because it is the accepted standard of care. Given the lack of information on treatment arms and newly-found association with gender and age, it is unclear how useful this study is.

The discrepancy in these studies could be attributed by small sample sizes (lack of power) as well as heterogeneity of tumors, patients and treatments [88]. Alternatively, given single polymorphisms have no significant associations after adjusting for multiple comparison, the effect of individual polymorphisms may be simply minimal to modest [57]. Using survival tree analysis and/or joint analysis of multiple variants, Wu et al. suggest there is a significant gene dosage effect and a polygenic analytical approach should be used to predict clinical outcomes.

Combining the above evidence, it seems that rs1695 is associated with favorable response to cisplatin and may be useful as a biomarker for predicting clinical outcomes on its own or in combination with other biomarkers—*i.e.*, as part of a polygenic predictive analysis for patients. There are several indications that variant rs1138272 may be associated with greater median survival; however, conclusive data are lacking. Both rs1695 and rs11138272 would benefit from further analysis with larger sample sizes and homogenous populations in terms of treatment regimen and histopathological tumor type are needed to conclude what role they play in cisplatin response in NSCLC patients.

GSTM and cisplatin sensitivity

Similar to the *GSTP* family of genes, the *GSTM* genes encode proteins involved in detoxification of xenobiotics. Its function with respect to NSCLC and cisplatin sensitivity is multifaceted as it is potentially a determinant of both susceptibility to NSCLC and response to cisplatin [96]. There are 5 genes in *GSTM* family: *GSTM1* through *GSTM5*. It is known that different proteins in the GST Mu (GSTM) family bind specifically to substrates such as the mutagen benzo[a]pyrene or the anticancer alkylating agent bis-chloroethylnitrosourea (BCNU) [96]. However, little has been done in studying the effect of *GSTM* expression on cisplatin sensitivity.

The little evidence available generally indicates *GSTM* expression does not have a significant effect on cisplatin. For example, three early studies found no link between *GSTM* expression and cisplatin resistance. Saburi et al. found no increase in *GSTM* expression in two cisplatin-resistant Chinese hamster ovary cell lines compared to their parent cell lines [97]. Townsend et al. showed that a breast cancer cell line overexpressing GSTM proteins exhibited no resistance to cisplatin, among a number of other drugs [43]. Waxman and colleagues monitored expression of different *GST* mRNAs in the livers of adult male rats and observed no change in *GSTM* expression in response to treatment with cisplatin [44]. However, a number of more recent studies have indicated that GSTM protein expression may have a significant association with cisplatin sensitivity. Watson et al. showed that there is a significant down-regulation of the *GSTM3* and *GSTM4* genes (4.84 and 3.13-fold decrease) in a cisplatin-resistant breast cancer cell line compared to the non-resistant parent cell line as measured by expression microarray analysis and real-time RT-PCR [98]. The same group of investigators confirmed these findings for *GSTM3* using the same cell line and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry [99]. In an intriguing meta-analysis of genome-wide association studies, Wheeler et al. showed that in 608 lymphoblastoid cell lines (LCLs) across seven panels that represent diverse world populations, rs10431718, a SNP within an eQTL targeting *GSTM1*, is significantly associated with cisplatin IC_{50} ($P = 0.017$), indicating that the regulation of the expression of *GSTM1* has a significant effect on cisplatin sensitivity [100]. There also exists compelling evidence from rational drug design studies of *GSTM1*'s importance in cisplatin sensitivity. One such study by Wang and colleagues found that by using a novel GST inhibitor which acts on *GSTM1,* sensitivity to cisplatin in a lung cancer cell line increased with a 2.7-fold decrease in IC_{50}. They further confirmed their findings by depleting those cells of *GSTM1* with a specific siRNA and observed a 1.4-fold decrease in cisplatin IC_{50} [45]. While some of the recent evidence is promising, it is still largely indirect and contradicts earlier direct studies. Therefore, more evidence is needed to elucidate the role of *GSTM* expression in cisplatin sensitivity.

Genes in the *GSTM* family are highly polymorphic. Four functional alleles have been observed in *GSTM1*, including *GSTM1**A, *GSTM1**B, *GSTM1**0 and *GSTM1**1×2. The first two correspond to catalytically-identical proteins (incorporating lysine and asparagine, respectively), while the latter two correspond to a deleted allele and a duplicated gene, respectively [101]. No known genetic polymorphisms had been reported for *GSTM2* and *GSTM5* yet, whereas *GSTM3* and *GSTM4* each have two distinct alleles (*GSTM3**A and *GSTM3**B; and *GSTM4**A and *GSTM4**B, respectively) [101]. The highly polymorphic nature has made *GSTM* genes the target of many studies. However, many of those studies have been focused on the relationship between *GSTM* genotype and susceptibility to cancer [101]. It is likely that *GSTM* genes play a role both in cancer susceptibility, by detoxifying potential carcinogen, and in drug sensitivity, by deactivating anticancer compounds.

The null genotype of *GSTM1* has been of particular interest as it occurs in roughly half of the human population with large variation across ethnic populations [102]. A meta-analysis of 90 studies from 1990 to 2009 of associations between 170 genetic polymorphisms and lung cancer outcome found that *GSTM1**0, the null genotype, was significantly associated with worse overall survival [103]. However, this conclusion was based on only two studies [104,105]. In particular, only one study had the majority of their patient population treated with cisplatin (70 out of 81 total patients), and the cisplatin treatment was in conjunction with etoposide. Furthermore, this study included 20 SCLC patients, which makes extrapolation to NSCLC patients difficult. More recent evidence has come to light, which indicates that the *GSTM1* null genotype is associated with improved response to platinum-based therapy [62]. Yang and Xian conducted a meta-analysis incorporating 455 NSCLC patients treated with platinum-based therapy with a known *GSTM1* genotype. They found that compared to the non-null genotype, the null genotype is significantly associated with good response (odds ratio = 1.77, 95% CI = 1.19−2.62). Nonetheless, it is worth noting that this association holds in East-Asian patients, but not among Caucasian patients [62].

Other *GSTM* polymorphisms have been less intensively studied vis-à-vis cisplatin sensitivity. Moyer et al. provided

one such study. They genotyped samples from 973 lung cancer patients (17% as SCLC) for SNPs from glutathione pathway related genes and found that rs560018, located on *GSTM4*, is significantly associated with improved survival ($P = 0.002$). They further found that in 100 LCLs treated with cisplatin, the same SNP is associated with cisplatin IC_{50} ($P = 0.019$) [63].

Evidence for both expression and genetic polymorphisms in the GSTM family associated with cisplatin sensitivity is currently lacking, but further investigation is warranted, especially with regard to the *GSTM1* null genotype.

Conclusion

In summary, we have discussed three representative pathways related to cisplatin sensitivity in NSCLC. Within the NER pathway, *ERCC1* expression appears to be a promising candidate for a cisplatin sensitivity biomarker. While expression of other genes may be similarly predictive of cisplatin sensitivity, current evidence is inconclusive. Within the copper transport pathway, there is varying degrees of evidence available for *CTR1*, *ATP7A* and *ATP7B* to support their use as biomarkers, but are a promising set of genes to consider. Current evidence is conflicting on the potential use of GST-related biomarkers. Looking ahead, we would propose larger and more powerful studies on the use of *ERCC1* as a cisplatin sensitivity biomarker as it is currently the most promising lead.

Competing interests

The authors have declared no competing interests.

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References

[1] WHO cancer factsheet. <http://www.who.int/mediacentre/factsheets/fs297/en/>; 2014 [accessed 25.05.14].
[2] American Cancer Society. Cancer facts and figures 2014. Atlanta: American Cancer Society; 2014.
[3] Yin JY, Huang Q, Zhao YC, Zhou HH, Liu ZQ. Meta-analysis on pharmacogenetics of platinum-based chemotherapy in non small cell lung cancer (NSCLC) patients. PLoS One 2012;7:e38150.
[4] National Cancer Institute. Non-small cell lung cancer treatment. <http://www.cancer.gov/cancertopics/pdq/treatment/non-small-cell-lung/healthprofessional/page10>; 2014 [accessed 25.05.14].
[5] Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014;64:9–29.
[6] Kuo MT, Chen HH, Song IS, Savaraj N, Ishikawa T. The roles of copper transporters in cisplatin resistance. Cancer Metastasis Rev 2007;26:71–83.
[7] van der Straaten T, Mullenders L, Guchelaar HJ. Pharmacogenetics of cancer and DNA repair enzymes. In: Vengrova S, editor. DNA repair and human health [internet]. Richardson: Intech; 2011. p. 597–612.
[8] Rabik CA, Dolan ME. Molecular mechanisms of resistance and toxicity associated with platinating agents. Cancer Treat Rev 2007;33:9–23.
[9] Huang JC, Zamble DB, Reardon JT, Lippard SJ, Sancar A. HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc Natl Acad Sci U S A 1994;91:10394–8.
[10] Fichtinger-Schepman AM, van der Veer JL, den Hartog JH, Lohman PH, Reedijk J. Adducts of the antitumor drug cis-diamminedichloroplatinum(ii) with DNA – formation, identification, and quantitation. Biochemistry 1985;24:707–13.
[11] Zamble DB, Mu D, Reardon JT, Sancar A, Lippard SJ. Repair of cisplatin-DNA adducts by the mammalian excision nuclease. Biochemistry 1996;35:10004–13.
[12] Kartalou M, Essigmann JM. Mechanisms of resistance to cisplatin. Mutat Res Fundam Mol Mech Mutagen 2001;478:23–43.
[13] Rosell R, Lord RV, Taron M, Reguart N. DNA repair and cisplatin resistance in non-small-cell lung cancer. Lung Cancer 2002;38:217–27.
[14] Furuta T, Ueda T, Aune G, Sarasin A, Kraemer KH, Pommier Y. Transcription-coupled nucleotide excision repair as a determinant of cisplatin sensitivity of human cells. Cancer Res 2002;62:4899–902.
[15] Rosell R, Taron M, Barnadas A, Scagliotti G, Sarries C, Roig B. Nucleotide excision repair pathways involved in Cisplatin resistance in non-small-cell lung cancer. Cancer Control 2003;10:297–305.
[16] De Silva IU, McHugh PJ, Clingen PH, Hartley JA. Defects in interstrand cross-link uncoupling do not account for the extreme sensitivity of ERCC1 and XPF cells to cisplatin. Nucleic Acids Res 2002;30:3848–56.
[17] Dabholkar M, Vionnet J, Bostickbruton F, Yu JJ, Reed E. Messenger-RNA levels of XPAC and ERCC1 in ovarian-cancer tissue correlate with response to platinum-based chemotherapy. J Clin Invest 1994;94:703–8.
[18] Takenaka T, Yoshino I, Kouso H, Ohba T, Yohena T, Osoegawa A, et al. Combined evaluation of Rad51 and ERCC1 expressions for sensitivity to platinum agents in non-small cell lung cancer. Int J Cancer 2007;121:895–900.
[19] Wang L, Wei J, Qian X, Yin H, Zhao Y, Yu L, et al. ERCC1 and BRCA1 mRNA expression levels in metastatic malignant effusions is associated with chemosensitivity to cisplatin and/or docetaxel. BMC Cancer 2008;8:97.
[20] Lord RV, Brabender J, Gandara D, Alberola V, Camps C, Domine M, et al. Low ERCC1 expression correlates with prolonged survival after cisplatin plus gemcitabine chemotherapy in non-small cell lung cancer. Clin Cancer Res 2002;8:2286–91.
[21] Cobo M, Isla D, Massuti B, Montes A, Sanchez JM, Provencio M, et al. Customizing cisplatin based on quantitative excision repair cross-complementing 1 mRNA expression: a phase III trial in non-small-cell lung cancer. J Clin Oncol 2007;25:2747–54.
[22] Olaussen KA, Dunant A, Fouret P, Brambilla E, Andre F, Haddad V, et al. DNA repair by ERCC1 in non-small-cell lung cancer and cisplatin-based adjuvant chemotherapy. N Engl J Med 2006;355:983–91.
[23] Fan W, Zhang HL, Wu XM. Enhancement effect of nucleotide excision repair gene xeroderma pigmentosum group A antisense RNA on sensitivity of human lung adenocarcinoma cell line A549 to cisplatin. Chin J Cancer 2005;24:403–7.

[24] Zhang Q, Wu JX, Wei YP, Hao JQ, Huang SY, Dong L. Effect of XPA expression on the chemotherapy sensitivity of A549/DDP cells. Chin J Cell Mol Immunol 2012;28:1182–4.
[25] Liu Y, Bernauer AM, Yingling CM, Belinsky SA. HIF1 alpha regulated expression of XPA contributes to cisplatin resistance in lung cancer. Carcinogenesis 2012;33:1187–92.
[26] Chen HH, Yan JJ, Chen WC, Kuo MT, Lai YH, Lai WW, et al. Predictive and prognostic value of human copper transporter 1 (hCtr1) in patients with stage III non-small-cell lung cancer receiving first-line platinum-based doublet chemotherapy. Lung Cancer 2012;75:228–34.
[27] Song IS, Savaraj N, Siddik ZH, Liu PM, Wei YJ, Wu CJ, et al. Role of human copper transporter Ctr1 in the transport of platinum-based antitumor agents in cisplatin-sensitive and cisplatin-resistant cells. Mol Cancer Ther 2004;3:1543–9.
[28] Lin XJ, Okuda T, Holzer A, Howell SB. The copper transporter CTR1 regulates cisplatin uptake in *Saccharomyces cerevisiae*. Mol Pharmacol 2002;62:1154–9.
[29] Ishida S, Lee J, Thiele DJ, Herskowitz I. Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc Natl Acad of Sci U S A 2002;99:14298–302.
[30] Li ZH, Qiu MZ, Zeng ZL, Luo HY, Wu WJ, Wang F, et al. Copper-transporting P-type adenosine triphosphatase (ATP7A) is associated with platinum-resistance in non-small cell lung cancer (NSCLC). J Transl Med 2012;10:21.
[31] Song L, Li Y, Li W, Wu S, Li Z. MiR-495 enhances the sensitivity of non-small cell lung cancer cells to platinum by modulation of copper transporting P-type adenosine triphosphatase A (ATP7A). J Cell Biochem 2014;115:1234–42.
[32] Nakayama K, Kanzaki A, Terada K, Mutoh M, Ogawa K, Sugiyama T, et al. Prognostic value of the Cu-transporting ATPase in ovarian carcinoma patients receiving cisplatin-based chemotherapy. Clin Cancer Res 2004;10:2804–11.
[33] Konkimalla VB, Kaina B, Efferth T. Role of transporter genes in cisplatin resistance. In Vivo 2008;22:279–83.
[34] Inoue Y, Matsumoto H, Yamada S, Kawai K, Suemizu H, Gika M, et al. ATP7B expression is associated with in vitro sensitivity to cisplatin in non-small cell lung cancer. Oncol Lett 2010;1:279–82.
[35] Nakagawa T, Inoue Y, Kodama H, Yamazaki H, Kawai K, Suemizu H, et al. Expression of copper-transporting P-type adenosine triphosphatase (ATP7B) correlates with cisplatin resistance in human non-small cell lung cancer xenografts. Oncol Rep 2008;20:265–70.
[36] Nakagawa K, Yokota J, Wada M, Sasaki Y, Fujiwara Y, Sakai M, et al. Levels of glutathione S transferase pi mRNA in human lung cancer cell lines correlate with the resistance to cisplatin and carboplatin. Jpn J Cancer Res 1988;79:301–4.
[37] Arai T, Yasuda Y, Takaya T, Yoshimi N, Ito H, Fujiwara H. Correlation between the immunohistochemical and mRNA expression of glutathione S-transferase-pi and cisplatin plus etoposide chemotherapy response in patients with untreated primary non-small cell lung cancer. Int J Oncol 1997;11:127–31.
[38] Arai T, Yasuda Y, Takaya T, Hayakawa K, Toshima S, Shibuya C, et al. Immunohistochemical expression of glutathione transferase-pi in untreated primary non-small-cell lung cancer. Cancer Detect Prev 2000;24:252–7.
[39] Hida T, Ariyoshi Y, Kuwabara M, Sugiura T, Takahashi T, Hosoda K, et al. Glutathione S-transferase pi levels in a panel of lung cancer cell lines and its relation to chemo-radiosensitivity. Jpn J Clin Oncol 1993;23:14–9.
[40] Hirano T, Kato H, Maeda M, Gong YB, Shou YN, Nakamura M, et al. Identification of postoperative adjuvant chemotherapy responders in non-small cell lung cancer by novel biomarker. Int J Cancer 2005;117:460–8.
[41] Allen TC, Granville LA, Cagle PT, Haque A, Zander DS, Barrios R. Expression of glutathione S-transferase pi and glutathione synthase correlates with survival in early stage non-small cell carcinomas of the lung. Hum Pathol 2007;38:220–7.
[42] Rosell R, Scagliotti G, Danenberg KD, Lord RV, Bepler G, Novello S, et al. Transcripts in pretreatment biopsies from a three-arm randomized trial in metastatic non-small-cell lung cancer. Oncogene 2003;22:3548–53.
[43] Townsend AJ, Tu CP, Cowan KH. Expression of human mu or alpha class glutathione S-transferases in stably transfected human MCF-7 breast cancer cells: effect on cellular sensitivity to cytotoxic agents. Mol Pharmacol 1992;41:230–6.
[44] Waxman DJ, Sundseth SS, Srivastava PK, Lapenson DP. Gene-specific oligonucleotide probes for alpha, mu, pi, and microsomal rat glutathione S-transferases: analysis of liver transferase expression and its modulation by hepatic enzyme inducers and platinum anticancer drugs. Cancer Res 1992;52:5797–802.
[45] Wang CH, Wu HT, Cheng HM, Yen TJ, Lu IH, Chang HC, et al. Inhibition of glutathione S-transferase M1 by new gabosine analogues is essential for overcoming cisplatin resistance in lung cancer cells. J Med Chem 2011;54:8574–81.
[46] Wachters FM, Wong LS, Timens W, Kampinga HH, Groen HJ. ERCC1, hRad51, and BRCA1 protein expression in relation to tumour response and survival of stage III/IV NSCLC patients treated with chemotherapy. Lung Cancer 2005;50:211–9.
[47] Booton R, Ward T, Ashcroft L, Morris J, Heighway J, Thatcher N. ERCC1 mRNA expression is not associated with response and survival after platinum-based chemotherapy regimens in advanced non-small cell lung cancer. J Thorac Oncol 2007;2:902–6.
[48] Glaysher S, Yiannakis D, Gabriel FG, Johnson P, Polak ME, Knight LA, et al. Resistance gene expression determines the in vitro chemosensitivity of non-small cell lung cancer (NSCLC). BMC Cancer 2009;9:1–17.
[49] Friboulet L, Olaussen KA, Pignon JP, Shepard FA, Tsao MS, Graziano S, et al. ERCC1 isoform expression and DNA repair in non-small cell lung cancer. N Engl J Med 2013;368:1101–10.
[50] Schneider JG, Farhadfar N, Sivapiragasam A, Geller M, Islam S, Selbs E. Commercial laboratory testing of excision repair cross-complementation group 1 expression in non-small cell lung cancer. Oncologist 2014;19:459–65.
[51] Stewart DJ. Tumor and host factors that may limit efficacy of chemotherapy in non-small cell and small cell lung cancer. Crit Rev Oncol Hematol 2010;75:173–234.
[52] Wei SZ, Zhan P, Shi MQ, Shi Y, Qian Q, Yu LK, et al. Predictive value of ERCC1 and XPD polymorphism in patients with advanced non-small cell lung cancer receiving platinum-based chemotherapy: a systematic review and meta-analysis. Med Oncol 2011;28:315–21.
[53] Wei HB, Hu J, Shang LH, Zhang YY, Lu FF, Wei M, et al. A meta-analytic review of ERCC1/MDR1 polymorphism and chemosensitivity to platinum in patients with advanced non-small cell lung cancer. Chin Med J (Engl) 2012;125:2902–7.
[54] Yu D, Shi J, Sun T, Du X, Liu L, Zhang X, et al. Pharmacogenetic role of ERCC1 genetic variants in treatment response of platinum-based chemotherapy among advanced non-small cell lung cancer patients. Tumor Biol 2012;33:877–84.
[55] Qin Q, Zhang C, Yang X, Zhu H, Yang B, Cai J, et al. Polymorphisms in XPD gene could predict clinical outcome of platinum-based chemotherapy for non-small cell lung cancer patients: a meta-analysis of 24 studies. PLoS One 2013;8:e79864.
[56] Xu X, Duan L, Zhou B, Ma R, Zhou H, Liu Z. Genetic polymorphism of copper transporter protein 1 is related to platinum resistance in Chinese non-small cell lung carcinoma patients. Clin Exp Pharmacol Physiol 2012;39:786–92.
[57] Wu X, Lu C, Ye Y, Chang J, Yang H, Lin J, et al. Germline genetic variations in drug action pathways predict clinical outcomes in advanced lung cancer treated with platinum-based chemotherapy. Pharmacogenet Genomics 2008;18:955–65.

[58] Sun N, Sun X, Chen B, Cheng H, Feng J, Cheng L, et al. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. Cancer Chemother Pharmacol 2010;65:437–46.
[59] Zhou F, Yu Z, Jiang T, Lv H, Yao R, Liang J. Genetic polymorphisms of GSTP1 and XRCC1: prediction of clinical outcome of platinum-based chemotherapy in advanced non-small cell lung cancer (NSCLC) patients. Swiss Med Wkly 2011;141:w13275.
[60] Booton R, Ward T, Heighway J, Ashcroft L, Morris J, Thatcher N. Glutathione S-transferase P1 isoenzyme polymorphisms, platinum-based chemotherapy, and non-small cell lung cancer. J Thorac Oncol 2006;1:679–83.
[61] Lu C, Spitz MR, Zhao H, Dong Q, Truong M, Chang JY, et al. Association between glutathione S-transferase pi polymorphisms and survival in patients with advanced nonsmall cell lung carcinoma. Cancer 2006;106:441–7.
[62] Yang Y, Xian L. The association between the GSTP1 A313G and GSTM1 null/present polymorphisms and the treatment response of the platinum-based chemotherapy in non-small cell lung cancer (NSCLC) patients: a meta-analysis. Tumor Biol 2014;35:6791–9.
[63] Moyer AM, Sun Z, Batzler AJ, Li L, Schaid DJ, Yang P, et al. Glutathione pathway genetic polymorphisms and lung cancer survival after platinum-based chemotherapy. Cancer Epidemiol Biomarkers Prev 2010;19:811–21.
[64] Wu XM, Fan W, Xu SW, Zhou YK. Sensitization to the cytotoxicity of cisplatin by transfection with nucleotide excision repair gene xeroderma pigmentosum group A antisense RNA in human lung adenocarcinoma cells. Clin Cancer Res 2003;9:5874–9.
[65] Rosenberg E, Taher MM, Kuemmerle NB, Farnsworth J, Valerie K. A truncated human xeroderma pigmentosum complementation group a protein expressed from an adenovirus sensitizes human tumor cells to ultraviolet light and cisplatin. Cancer Res 2001;61:764–70.
[66] Gately DP, Howell SB. Cellular accumulation of the anticancer agent cisplatin: a review. Br J Cancer 1993;67:1171–6.
[67] Kim ES, Lee JJ, He G, Chow CW, Fujimoto J, Kalhor N, et al. Tissue platinum concentration and tumor response in non-small-cell lung cancer. J Clin Oncol 2012;30:3345–52.
[68] Zhou B, Gitschier J. HCTR1: a human gene for copper uptake identified by complementation in yeast. Proc Natl Acad Sci U S A 1997;94:7481–6.
[69] Safaei R, Howell SB. Copper transporters regulate the cellular pharmacology and sensitivity to Pt drugs. Crit Rev Oncol Hematol 2005;53:13–23.
[70] Holzer AK, Manorek GH, Howell SB. Contribution of the major copper influx transporter CTR1 to the cellular accumulation of cisplatin, carboplatin, and oxaliplatin. Mol Pharmacol 2006;70:1390–4.
[71] Zisowsky J, Koegel S, Leyers S, Devarakonda K, Kassack MU, Osmak M, et al. Relevance of drug uptake and efflux for cisplatin sensitivity of tumor cells. Biochem Pharmacol 2007;73:298–307.
[72] Larson CA, Blair BG, Safaei R, Howell SB. The role of the mammalian copper transporter 1 in the cellular accumulation of platinum-based drugs. Mol Pharmacol 2009;75:324–30.
[73] Larson CA, Adams PL, Jandial DD, Blair BG, Safaei R, Howell SB. The role of the N-terminus of mammalian copper transporter 1 in the cellular accumulation of cisplatin. Biochem Pharmacol 2010;80:448–54.
[74] Kalayda GV, Wagner CH, Jaehde U. Relevance of copper transporter 1 for cisplatin resistance in human ovarian carcinoma cells. J Inorg Biochem 2012;116:1–10.
[75] Ishida S, McCormick F, Smith-McCune K, Hanahan D. Enhancing tumor-specific uptake of the anticancer drug cisplatin with a copper chelator. Cancer Cell 2010;17:574–83.
[76] Fu S, Naing A, Fu C, Kuo MT, Kurzrock R. Overcoming platinum resistance through the use of a copper-lowering agent. Mol Cancer Ther 2012;11:1221–5.
[77] Ivy KD, Kaplan JH. A re-evaluation of the role of hCTR1, the human high-affinity copper transporter, in platinum-drug entry into human cells. Mol Pharmacol 2013;83:1237–46.
[78] Hall MD, Okabe M, Shen DW, Liang XJ, Gottesman MM. The role of cellular accumulation in determining sensitivity to platinum-based chemotherapy. Annu Rev Pharmacol Toxicol 2008;48:495–535.
[79] Wee NK, Weinstein DC, Fraser ST, Assinder SJ. The mammalian copper transporters CTR1 and CTR2 and their roles in development and disease. Int J Biochem Cell Biol 2013;45:960–3.
[80] Blair BG, Larson CA, Safaei R, Howell SB. Copper transporter 2 regulates the cellular accumulation and cytotoxicity of Cisplatin and Carboplatin. Clin Cancer Res 2009;15:4312–21.
[81] Huang CP, Fofana M, Chan J, Chang CJ, Howell SB. Copper transporter 2 regulates intracellular copper and sensitivity to cisplatin. Metallomics 2014;6:654–61.
[82] Ohrvik H, Thiele DJ. The role of Ctr1 and Ctr2 in mammalian copper homeostasis and platinum-based chemotherapy. J Trace Elem Med Biol 2014. http://dx.doi.org/10.1016/j.jtemb.2014.03.006.
[83] Vulpe C, Levinson B, Whitney S, Packman S, Gitschier J. Isolation of a candidate gene for Menkes disease and evidence that it encodes a copper-transporting ATPase. Nat Genet 1993;3:7–13.
[84] Bull PC, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting p-type ATPase similar to the Menkes gene. Nat Genet 1993;5:327–37.
[85] Samimi G, Varki NM, Wilczynski S, Safaei R, Alberts DS, Howell SB. Increase in expression of the copper transporter ATP7A during platinum drug-based treatment is associated with poor survival in ovarian cancer patients. Clin Cancer Res 2003;9:5853–9.
[86] Samimi G, Safaei R, Katano K, Holzer AK, Rochdi M, Tomioka M, et al. Increased expression of the copper efflux transporter ATP7A mediates resistance to cisplatin, carboplatin, and oxaliplatin in ovarian cancer cells. Clin Cancer Res 2004;10:4661–9.
[87] Komatsu M, Sumizawa T, Mutoh M, Chen ZS, Terada K, Furukawa T, et al. Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. Cancer Res 2000;60:1312–6.
[88] Hildebrandt MA, Gu J, Wu X. Pharmacogenomics of platinum-based chemotherapy in NSCLC. Expert Opin Drug Metab Toxicol 2009;5:745–55.
[89] Fukushima-Uesaka H, Saito Y, Maekawa K, Kurose K, Sugiyama E, Katori N, et al. Genetic polymorphisms of copper- and platinum drug-efflux transporters ATP7A and ATP7B in Japanese cancer patients. Drug Metab Pharmacokinet 2009;24:565–74.
[90] Anttila S, Hirvonen A, Vainio H, Husgafvel-Pursiainen K, Hayes JD, Ketterer B. Immunohistochemical localization of glutathione S-transferases in human lung. Cancer Res 1993;53:5643–8.
[91] Terrier P, Townsend AJ, Coindre JM, Triche TJ, Cowan KH. An immunohistochemical study of pi-class glutathione S-transferase expression in normal human tissue. Am J Pathol 1990;137:845–53.
[92] Peklak-Scott C, Smitherman PK, Townsend AJ, Morrow CS. Role of glutathione S-transferase P1–1 in the cellular detoxification of cisplatin. Mol Cancer Ther 2008;7:3247–55.
[93] Tew KD. Glutathione-associated enzymes in anticancer drug-resistance. Cancer Res 1994;54:4313–20.
[94] Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relation-

ship to lung tissue enzyme activity and population frequency distribution. Carcinogenesis 1998;19:275–80.
[95] Allan JM, Wild CP, Rollinson S, Willett EV, Moorman AV, Dovey GJ, et al. Polymorphism in glutathione S-transferase P1 is associated with susceptibility to chemotherapy-induced leukemia. Proc Natl Acad Sci U S A 2001;98:11592–7.
[96] Hayes JD, Pulford DJ. The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995;30:445–600.
[97] Saburi Y, Nakagawa M, Ono M, Sakai M, Muramatsu M, Kohno K, et al. Increased expression of glutathione S-transferase gene in *cis*-diamminedichloro-platinum(ii)-resistant variants of a Chinese hamster ovary cell line. Cancer Res 1989;49:7020–5.
[98] Watson MB, Lind MJ, Smith L, Drew PJ, Cawkwell L. Expression microarray analysis reveals genes associated with *in vitro* resistance to cisplatin in a cell line model. Acta Oncol 2007;46:651–8.
[99] Smith L, Welham KJ, Watson MB, Drew PJ, Lind MJ, Cawkwell L. The proteomic analysis of cisplatin resistance in breast cancer cells. Oncol Res 2007;16:497–506.
[100] Wheeler HE, Gamazon ER, Stark AL, O'Donnel PH, Gorsic LK, et al. Genome-wide meta-analysis identifies variants associated with platinating agent susceptibility across populations. Pharmacogenomics J 2013;13:35–43.
[101] Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. Pharmacology 2000;61:154–66.
[102] Lin HJ, Han CY, Bernstein DA, Hsiao W, Lin BK, Hardy S. Ethnic distribution of the glutathione transferase mu-1-1 (GSTM1) null genotype in 1473 individuals and application to bladder-cancer susceptibility. Carcinogenesis 1994;15:1077–81.
[103] Horgan AM, Yang B, Azad AK, Amir E, John T, Cescon DW, et al. Pharmacogenetic and germline prognostic markers of lung cancer. J Thorac Oncol 2011;6:296–304.
[104] Sweeney C, Nazar-Stewart V, Stapleton PL, Eaton DL, Vaughan TL. Glutathione S-transferase M1, T1, and P1 polymorphisms and survival among lung cancer patients. Cancer Epidemiol Biomarkers Prev 2003;12:527–33.
[105] Gonlugur U, Pinarbasi H, Gonlugur TE, Silig Y. The association between polymorphisms in glutathione S-transferase (GSTM1 and GSTT1) and lung cancer outcome. Cancer Invest 2006;24:497–501.