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
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 cross-links), 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]. 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 Dubholkar 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

| 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]   |
| ATP7A     | ↓                                           | Clinical study conducted in 89 patients  | [30]   |
| ATP7B     | ↓                                           | Clinical study conducted in 104 patients | [32]   |
| GSTP1     | ↓                                           | 60 human tumor cell lines including NSCLC | [33,34] |
| STM1      | ↔                                           | Cell lines, tumor samples and clinical study tracking expression and survival | [36-42] |

*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.*
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 when 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).

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 | 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 | Clinical study in 973 lung cancer patients [63] |
|           |           | G/G genotype associated with decreased sensitivity | 100 lymphoblastoid cell lines [63] |

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 SLC31A1) 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
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) [36]. 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 isomop 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 IC50 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 IC50 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 IIIIB 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 rs1138272 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-chloroethyl nitrosourea (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 over-expressing 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 IC50 ($P = 0.017$), 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 IC50. They further confirmed their findings by depleting those cells of GSTM1 with a specific siRNA and observed a 1.4-fold decrease in cisplatin IC50 [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 GSTM3 yet, whereas GSTM3 and GSTM4 each have two distinct alleles (GSTM1*A and GSTM1*B; and GSTM1*A and GSTM1*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 IC50 (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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