Research Highlights

Candidate gene pharmacogenetics of anticancer agents

In the field of cancer chemotherapy, the clinical study of genetic contribution to treatment effect is complicated by the disease characteristics, concurrent therapy/type of treatment regimen, mode of administration, prior treatment,

patient ethnic population, phenotype definition (e.g., good/poor response), genotyping methods and study sample size. In this Research Highlights article, we will discuss several recent clinical pharmacogenetic studies.

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A *DPYD* coding region SNP shows strong association with fluoropyrimidine-related toxicity

Evaluation of: Gross E, Busse B, Riemenschneider M *et al.*: Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PLoS One* 3(12), E4003 (2008).

Fluoropyrimidine drugs such as 5-fluorouracil (5-FU) or capecitabine were commonly used in treating gastroesophageal, colorectal and breast cancers [1]. Despite their clinical benefit, these drugs are associated with frequent gastrointestinal and hematological toxicities [2], which often lead to treatment discontinuation. Due to its function as an initial and rate-limiting enzyme in the catabolism of pyrimidines, dihydropyrimidine dehydrogenase (DPD, encoded by the *DPYD* gene) deactivates more than 80% of administered standard doses of 5-FU. Some *DPYD* alleles (e.g., *IVS14 +1 G>A* [*DPYD**2A, rs3918290] or 1679T>G [I560S, *DPYD**13, rs55886062]) have been associated with decreased DPD activity [3]. These SNPs have also been found to associate with grade IV neutropenia [4] and severe mucositis and leucopenia after 5-FU treatment [5].

Gross *et al.* evaluated the entire *DPYD* coding region and compared genotype frequencies between Caucasian cancer patients

with good (n = 89) and poor (n = 39) tolerance of a fluoropyrimidine-based chemotherapy regimen [1]. They identified a strong association between fluoropyrimidine-related grade III and IV toxicity and a nonsynonymous polymorphism c.496A>G (p.Met166Val, rs2297595) within the *DPYD* gene. This finding was further replicated in a second cohort of 53 cancer patients who experienced an acute adverse drug reaction.

A deleterious mutation (rs3918290), that has been suggested to associate with fluoropyrimidine-induced toxicity, showed very low minor allele frequency (MAF) in this study population. This was further confirmed by genotyping additional 157 healthy individuals. However, the rs2297595 genetic contribution identified in this study has a MAF of 0.08 in patients with good response or in a healthy population, and a greater than 0.20 MAF in patients who develop severe toxicity. Furthermore, only 5.4% of patients who developed toxicity carry the rs3918290 mutation, while 43% are carriers of the rs2297595 mutation. The mechanism by which this rs2297595 SNP affects fluoropyrimidine-induced toxicity remains to be evaluated.

Interestingly, rs2297595's genetic contribution to 5-FU based agent-induced toxicity was only evident in gastroesophageal and breast cancer patients; while this association did not reach statistical significance

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in colorectal carcinoma patients. This could be due to the limited sample size for colorectal cancers included in this study, or as the authors suggested the presence of additional drugs.

Furthermore, even though rs2297595 showed a significant contribution to drug induced toxicity with 56.9% calculated attributable risk for the variant allele, this association only explained portion of the drug-toxicity variation. Using genome-wide association to identify additional candidate genes was warranted.

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Genetic polymorphisms in *XRCC1* may affect response to platinum-based chemotherapy in advanced non-small-cell lung cancer patients

Evaluation of: Sun X, Li F, Sun N *et al.*: Polymorphisms in *XRCC1* and *XPG* and response to platinum-based chemotherapy in advanced non-small-cell lung cancer patients. *Lung Cancer* (2009) (Epub ahead of print).

Platinum-based chemotherapeutics are commonly used in treating advanced non-small-cell lung cancer (NSCLC); however, less than 30% of patients respond to therapy. To investigate the potential genetic contribution to platinum agent response in a Chinese population, Sun *et al.* focused on two candidate genes that are involved in the DNA repair pathway [1]. These genes are xeroderma pigmentosum group G (*XPG*) and x-ray repair cross complementing group 1 (*XRCC1*). Two SNPs were studied in each of these genes. A total of 82 stage IV NSCLC patients who underwent first-time platinum-based chemotherapy were included

in this study. Complete response and partial response were considered good responders; while stable disease and progressive disease were classified as poor responders. χ^2 tests showed significant genotype distribution differences between good and poor responders for *XRCC1* 194 Arg/Trp and *XPG* 46His His SNPs. Logistic regression showed that patients who are heterozygote carriers of *XRCC1* Trp194Arg have a better therapeutic response to platinating agent treatment.

Giachino *et al.* found a significant association between another *XRCC1* SNP (Arg399Gln) and grade III–IV clinical toxicity in NSCLC and small-cell lung carcinoma patients who underwent cisplatin treatment [2]. This particular SNP genotype did not show association with platinum treatment response in this study. The discrepancy may be due to different ethnic populations, different phenotype evaluated (response vs toxicity) or both.

The genetic effects attributed to platinating agent treatment response were

fairly small in this study. Since tumor size, lymph node and distant metastases status are all critical factors for NSCLC prognosis, it is questionable whether genetic predictors identified in this study will remain significant when performing multivariable analysis including clinical predictors. Furthermore, the phenomenon that only heterozygote carriers of variant alleles appear to be associated with treatment outcomes begs for a plausible explanation of the mechanism involved.

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A *GSTP1* SNP may affect thiotepa and tepa pharmacokinetics

Evaluation of: Ekhardt C, Doodeman V, Rodenhuis S *et al.*: Polymorphisms of drug-metabolizing enzymes (*GST*, *CYP2B6* and *CYP3A*) affect the pharmacokinetics of thiotepa and tepa. *Br. J. Clin. Pharmacol.* 67(1), 50–60 (2008).

Thiotepa is an alkylating anticancer agent often co-administered with other anticancer agents in high-dose chemotherapy regimens for the treatment of advanced or metastatic breast, ovarian and testis tumors [1]. Large interindividual variability has been observed for both thiotepa and tepa (a main metabolite of thiotepa) induced toxicities and their clearance. Furthermore, systemic exposure to thiotepa and tepa was reported to associate with toxicity. Thiotepa is metabolized by the cytochrome P450 (CYP) enzymes, namely *CYP2B6* and *CYP3A4*, to tepa. Both thiotepa and tepa are conjugated to glutathione, which is catalyzed by glutathione *S*-transferase (*GST*) isoenzymes A1–1 and P1–1. Ekhardt *et al.* evaluated genetic polymorphisms in these thiotepa/tepa metabolism enzymes for their effects on

thiotepa and tepa pharmacokinetics [1]. In this study, 124 Caucasian patients receiving a high-dose regimen consisting of cyclophosphamide, thiotepa and carboplatin were included. Genotyping of 11 SNPs was conducted through sequencing. Patients' plasma concentrations of thiotepa and tepa were determined using gas chromatography and liquid chromatography-mass spectrometry/mass spectrometry methods. The authors obtained population pharmacokinetic data of thiotepa and tepa through nonlinear mixed-effects modeling. SNP effects were first interrogated within each enzyme by performing association tests between allelic variants and elimination of pharmacokinetic parameters. The significant SNPs from each enzyme were then combined and underwent a backward selection process to identify a group of SNPs that are important for thiotepa/tepa clearance.

The *CYP2B6* C1459T, *CYP3A4**1B, *CYP3A5**3, *GSTA1* (C-69T, G-52A) and *GSTP1* C341T polymorphisms were found to have a significant effects on clearance of thiotepa or tepa. Although significant, most effects were small compared with large interindividual variability in

thiotepa and tepa clearance, with the exception of *GSTP1* C341T. This SNP increased noninducible thiotepa clearance by 52% and decreased tepa clearance by 32%. A simulation test showed an increase in combined thiotepa and tepa exposure of 45% in homozygous variant carriers compared with those of wild-type, which may be clinically relevant.

The findings from this study were complicated by the co-administration of cyclophosphamide, which may induce *CYP2B6* and *CYP3A* expression and affect their activity. This paper addressed the issue by introducing an inducible thiotepa clearance route to their population model. It will be worthwhile to investigate whether the genetic polymorphisms identified in the study remain significant predictors of thiotepa and tepa pharmacokinetic when certain modeling parameters were modified.

Reference

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Allele-specific inhibition, a potential new therapeutic strategy in cancer treatment

Evaluation of: Mook O, Baas F, Wissel MD, Fluiter K: Allele-specific cancer cell killing *in vitro* and *in vivo* targeting a single-nucleotide polymorphism in *POLR2A*. *Cancer Gene Ther.* (2009) (Epub ahead of print).

Many chemotherapeutic agents are cytotoxic to both tumor and normal tissues, which results in severe adverse effects. To design treatment regimens that are

specific to tumor tissue with minimal effect on normal tissues has always been the goal of cancer therapy. To this end, a novel therapeutic strategy termed allele-specific inhibition (ASI) has been developed. ASI therapy is based on the fact that cancer cells carry large regions of loss-of-heterozygosity (LOH). This unique cancer characteristic makes it a potential target for RNA interference-based therapy since many of the LOH regions contain genes essential for cell

survival. Furthermore, genetic variations (e.g., SNPs) have been found in these genes related to cell survival. An ASI can be designed when an essential gene is located in a LOH region and contains heterozygous SNPs in normal cells. The rationale is that cancer cells that have only one allele form of the essential gene will be sensitive to inhibition; while normal heterozygous cells containing both allele forms of the gene will be unaffected.

The large subunit of RNA polymerase II (*POLR2A*), a gene located in close proximity to the tumor suppressor gene *p53*, frequently shows LOH in cancer cells. It also contains heterozygous SNPs in normal cells. Mook *et al.* designed six siRNA duplexes (each has one or two mismatches) targeted to a *POLR2A* C/T SNP (position 2673) region [1]. These siRNAs were delivered to a pancreatic cancer cell line MiaPaca II (T genotype)

and a prostate cancer cell line 15PC3 (C genotype) to detect the sequence-specific inhibition of *POLR2A*. It was shown *in vitro* that siRNA could selectively inhibit *POLR2A* expression at both the mRNA and protein level depending on its genotype and the relative position of the SNP in its siRNA. This allele-specific inhibition on gene expression did translate to cell growth inhibition *in vitro*. Furthermore, the nude mice tumor xenograft model also showed tumor growth inhibition; however, the *in vitro* selected genotype-specific siRNA lost its

specificity *in vivo*. This study demonstrated that siRNA-based ASI is a potential therapeutic application to inhibit tumor growth by targeting a SNP in genes located in LOH regions of cancer cells. This may be a new approach to develop genotype-specific nucleic acid drugs.

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