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Relationships between genetic polymorphisms and transcriptional profiles for outcome prediction in anticancer agent treatment

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In the era of personal genomics, predicting the individual response to drug-treatment is a challenge of biomedical research. The aim of this study was to validate whether interaction information between genetic and transcriptional signatures are promising features to predict a drug response. Because drug resistance/susceptibilities result from the complex associations of genetic and transcriptional activities, we predicted the inter-relationships between genetic and transcriptional signatures. With this concept, captured genetic polymorphisms and transcriptional profiles were prepared in cancer samples. By splitting ninety-nine samples into a trial set (n = 30) and a test set (n = 69), the outperformance of relationship-focused model (0.84 of area under the curve in trial set, $P = 2.90 \times 10^{-4}$) was presented in the trial set and validated in the test set, respectively. The prediction results of modeling show that considering the relationships between genetic and transcriptional features is an effective approach to determine outcome predictions of drug-treatment. [BMB reports 2010; 43(12): 836-841]

INTRODUCTION

Developing a high-throughput technology for genomic and transcriptomic analysis may help select drug treatments based on the molecular signatures of disease samples and bring us closer to an era of personal genomics (1). Because cancers are lethal and heterogeneous diseases (2), various attempts have been discussed for the targeted treatment of anticancer agents, such as identifying individual variations (or resistances) to chemotherapy (3, 4). Although tumor resection followed by the administration of anticancer agent is beneficial, predicting individual differences in the drug response remains a critical issue due to the severe results of anticancer treatment (2, 5).

Previous gene expression studies investigated functional understandings for the heterogeneous drug susceptibilities (survival times) after treatment with anticancer agents (6, 7). Others assessing the transcriptional signature of cancer tissues suggested that transcriptomic analysis would be helpful for predicting relapse after the administration of anticancer agents (8). Similarly, recent efforts to find genetic associations and indicative loci for the susceptibility to anticancer agents suggested that genetic variants accounted for differences in drug-response and cytotoxicity due to the functional alteration of enzymes that metabolize chemical agents, such as taxane and platinum-base agents (9, 10). However, transcriptional signatures provide snap-shot information, while the mechanisms driving individual differences in the response of anticancer agents are very complex. Moreover, no genetic polymorphisms have been conclusively verified (11), and the functional role of the identified genetic polymorphisms has been presented by independent studies (12) without direct evidence, such as *in vivo* transcriptional alterations.

Because research related to the susceptibility of anticancer agents using either genetic or transcriptomic approaches might present limited insight for variation in the chemoresponse, integrative bioinformatics approaches might facilitate predicting heterogeneous survival. Therefore, genetic analyses including transcriptional alterations have received increasing attention lately (13) with promising results. For example, transcriptional contributions of genetic polymorphisms (SNPs, single nucleotide polymorphisms) for cytotoxicity due to anticancer agents were listed using human cell lines (12). These results from genomic transcript studies manifest that the development of a prediction model with "inter-relations" between genetic and transcriptional features might help predict individual variations in the response to chemical anticancer treatments. For the proof of this biological intuition, a "prediction with a relationship of captured signatures", presentation of the prediction model which includes the value of inter-relationships is demanded.

Here, we predicted individual variation in the anticancer

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drug responses of cancer samples using a prediction model which consists of the inter-relationships between genetic polymorphisms and transcriptional profiles. The stochastic analysis was conducted to investigate promising genetic and transcriptomic signature markers including their inter-relationships. For this analysis, genetic polymorphisms and expression signatures were pairwise profiled for prepared ninety-nine cancer samples enrolled in the consortium of The Cancer Genome Atlas (TCGA). Samples were randomly split into trial and test sets to validate our predictions. The identified promising set of genetic polymorphisms, transcriptional profiles and novel features of the inter-relationships were introduced into our prediction model, using a trial set of samples (n = 30). The value of a developed relationship-focused approach was demonstrated by comparing the prediction performances of our model with other prediction scenarios conducted without consideration of an inter-relationship. With the prospective performance of our prediction model (0.84 of area under the curve [AUC]), this predictability was also validated in the remaining test set of samples.

RESULTS

Identified candidates of indicative markers in the trial set

Fig. 1 presents the overview of procedure for the marker selection and our prediction concept. With a threshold P-value (<0.05 with FDR), we identified genetic polymorphisms (Gv) and transcriptional profiles (Ts) in the anticancer agent-related pathways as indicative markers for survival after the administration of anticancer drugs (platinum and taxane agents) with a trial set of ovarian cancer (OV) samples (n = 30). To our knowledge, whereas previous studies reported the taxane-base anticancer agent efflux functions of ABCC1 and NR1I2 (14, 15), the association of their genetic variations to the individual variation of general treatment of OV (platinum-taxane) has been firstly suggested in our study. The transcriptional contribution of CYPA5 was identified in our study meanwhile genetic association of CYPA5 was taxane-base drug clearance with OV tissues (16) ($P = 6.12 \times 10^{-3}$, supplemental Table 1). Identified genetic polymorphisms and transcriptional profiles were utilized to predict individual outcomes of anticancer drug treatment with OV samples as candidates of indicative markers. Ts-, Gv-, and GT-prediction was performed, as four different prediction scenarios were simulated to highlight the power of our relationship-focused approach with the GTI-prediction model. Supplemental Table 1 presents the optimized candidate features for each prediction scenario. Five genetic polymorphisms and two transcriptional profiles of the taxane-base drug related pathway remained in our GTI-prediction model, including five features for their inter-relationship (Fig. 2 and supplemental Table 1, chi- square goodness-of-fit: $P = 2.90 \times 10^{-4}$). Although the genetic polymorphisms and transcriptional profiles of the platinum-base drug related pathway were tested in our prediction scenarios, inappropriate performance resulted (averaged differences between predicted and observed survival times = 408 days). Thus, prediction with members of the taxane related pathway were utilized in a further analysis to validate the trial set (n = 30), test set (n = 69), and excluded set (n = 6).

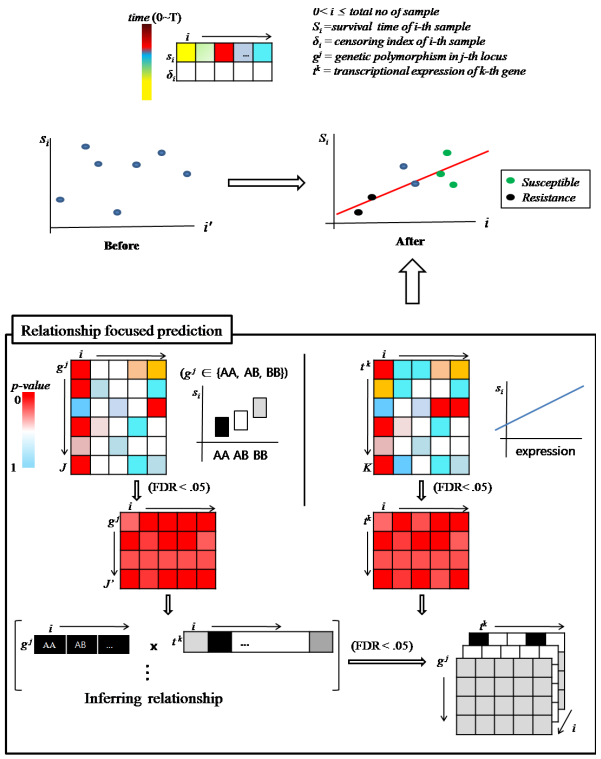

Fig. 1. Overview of the individual difference predictions after the administration of anticancer agents.

Prediction of individual outcomes after the administration of anticancer agents

We compared the accuracy of various prediction scenarios utilizing different categories of indicative markers to validate the value of a relationship-focused approach. In summary, the outperformance of the GTI-model suggested an advantage of our relationship-focused modeling, while Gv-, and Ts-prediction represented the performance of genetic association and transcriptional studies, and GT-prediction suggested limited accuracy for the integrative approaches without the concept of feature relationships (supplemental Table 1). Gv- and Ts-predictions were conducted using either genetic or transcriptional features, whereas GT-prediction was completed using both without relationship values. The major difference between GT- and GTI-prediction was the inter-relationship value, as described in Methods. Three different measures were incorporated to compare predictability with the trial set (n = 30): (1) Somer's Dxy correlation (17) of observed and predicted survival variations, (2) chi-square goodness-of-fit, and (3) AUC of survival

ROC (receiver operating characteristic) to determine the performance of the prediction models (18). As presented in Fig. 3a, the correlation of observed vs. predicted individual variations after the administration of anticancer agents by our GTI-prediction was higher than all the correlations from other scenarios with significant fitness ($P = 2.9 \times 10^{-4}$ for GTI-prediction vs. 1.20×10^{-2} for Gv-, 2.10×10^{-2} for Ts-, and 2.10×10^{-2} in GT-prediction). Fig. 3b shows that the AUC of the survival ROC for our GTI-prediction was higher than that of other scenarios (0.84 in GTI-prediction vs. 0.61, 0.38, and 0.35). The higher AUC value indicates improved accuracy, such as 1.00 of AUC for 100% predictability, and 0.84 of AUC for 84% accuracy. Thus, a higher AUC value for GTI-prediction denotes improved accuracy compared to the other predictions (Fig. 3a, b). To summarize, all different comparison panels consistently showed that the GTI-prediction established with the inter- relationship concept was superior to other prediction scenarios, suggesting that the information on inter-relationships between genetic polymorphisms and transcriptional profiles contributed to successful prediction for the outcome variation after anticancer agent treatment.

While we prepared candidate markers and successfully predicted outcome variations in a trial set, the issue of prediction advantage with trial set dependency and the random effect of a larger number of markers for GTI-prediction still remained. To solve these issues, we justified the superiority of the GTI-prediction model based on an independent test set of sixty-nine samples not utilized for indicative marker selection and modeling. As shown in Fig. 3d, the AUC of the survival ROC for the independent test set showed that it was appropriate compared to other prediction scenarios (0.61 vs. 0.39, 0.4, 0.51). However, the relevance of the prediction declined in the excluded OV sample set, which underwent another anticancer agent treatment (Fig. 3c). Favorable performance on the independent test set and limited performances on the excluded set showed that the outperformance of the GTI-prediction was mainly derived from the power of the inter-relationships and not by random results of the trial set. Thus, further application of the proposed concept, prediction using a feature relationship, will enhance the identification of individual differences after anticancer drug treatment. Focusing on the relationship between genetic polymorphism and transcrip-

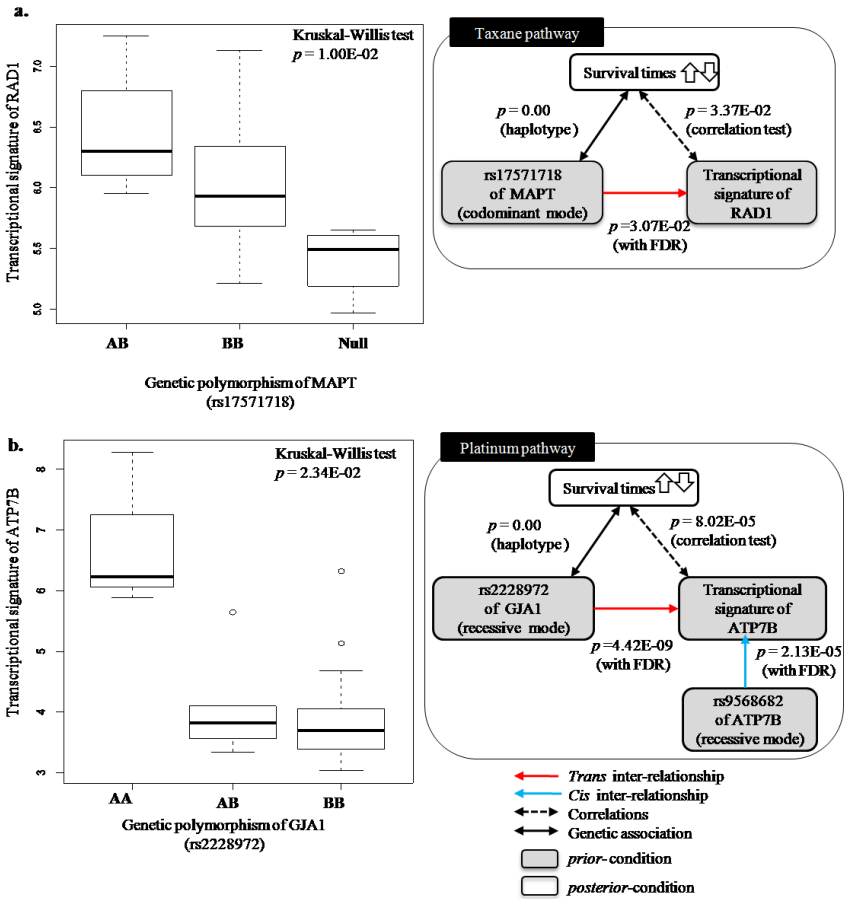

Fig. 2. Example of an inter-relationship between genetic polymorphisms and transcriptional profiles. The definition for the arrows in the right panels is denoted at right below. (a) Left panel shows RAD1 transcriptional alterations with a MAPT1 genotype difference (rs17571718). Right panel present the results of each step of the relationship analysis for the case in the left panel. (b) Left panel presents the GJA1 genetic contribution (rs2228971) for ATP7B expression, and both are members of the platinum pathway.

tional profile, we proposed a prediction model for individual variations in anticancer drug treatment with outperformed predictability.

DISCUSSION

Using bioinformatic approaches, we successfully predicted the individual variation in the administration of anticancer agents based on the inter-relationship of genetic polymorphism and transcriptional profile (0.84 of AUC). The proposed GTI-prediction identified the importance of the inter-relationship between genetic and transcriptional features using fair comparisons with various prediction scenarios in a trial set and a larger test set, respectively. Our hypothesis that the relationships of genetic polymorphism and transcriptional profile are true predictors of drug responses was validated by comparing the prediction results without considering the marker relationships (23-49% improvement). In biological network research, identifying interactomes in genomic and transcriptomic signatures is a promising route to describe biological phenomena. In the present research, we validated that the relationships between genomic and transcriptomic features are useful markers to predict the outcome of anticancer treatment. Predictions using relationships is a novel concept for the broad application to bioinformatic analysis.

Moreover, the identified relationships between genetic and transcriptional signatures in the GTI-prediction model indicate promising avenues for further interactome chemoresponse research, including taxane drug efflux (CYP3A5 and ABCC1 (19)), DNA excision repair (RAD1), and tubulin stabilization (MAPT (20)). For example, the ATP7B transcriptional profile is related with the GJA1 genetic polymorphism (rs2228972), with a significant P value (4.42×10^{-9} with FDR, Fig. 2). With the knowledge of ATP7B in pharmGKB, platinum agent efflux, GTI-prediction can indicate the underlying ATP7B expression mechanism as a *trans*-interaction over the prediction of survival outcomes. Because the GTI-prediction was validated using Caucasian samples, the reproducibility of our model should be determined for different ethnic groups. In a previous attempt, the cytotoxicity related transcriptional profiles following treatment with platinum agents were associated with genetic variations based on ethnic background (12). While epigenetic fea-

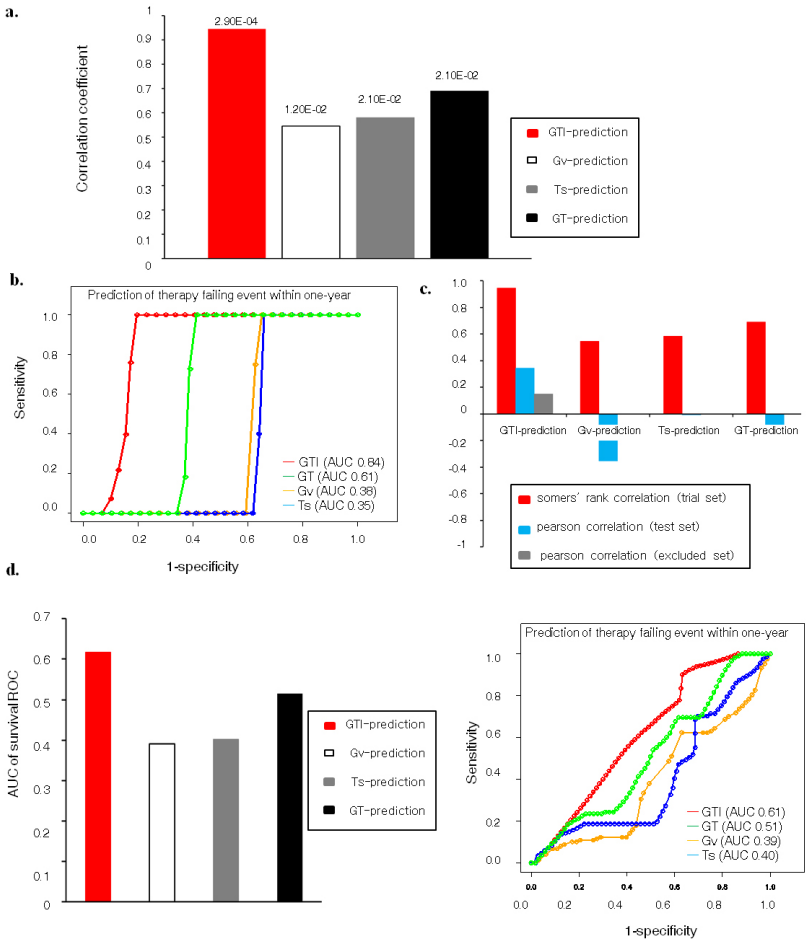

Fig. 3. Performance and predictability analysis. We compared the performance of the Gv-, Ts-, and GT-prediction, scenarios using the GTI-prediction. (a) A panel of Somers' Dxy correlations between predicted and observed survival variation after anticancer agent treatment, and P values for the chi- square goodness of fit. (b) Survival receiver operating characteristic (ROC) curve for the analyzed predictions in the trial set. (c) Correlations between the observed and predicted survival variation in the trial, independent test, and excluded sets. Pearson's correlation analysis was performed with the deceased case only. (d) Left panel denotes survival ROC area under the curve values for each prediction in the independent test set. Right panel shows survival ROCs with the independent test set.

tures, such as methylation of promoter region, also affect transcriptional profiles (21), we focused on the transcriptional alteration within a given genetic condition to prevent sample stratification by age-dependent methylations in prepared samples (age range, 35-83.5 years). Therefore, our prediction model may present different indicative markers for the relationship between genetic and transcriptional profiles in a further study with various TCGA phase II ethnic groups.

With the efforts of the TCGA project for the paired profiling of molecular signatures in various categories, such as genotype, expression profile, DNA methylation, miRNA and somatic mutations, we proposed a relationship focusing on prediction, with significant validation of predictability using genetic variations and transcriptional signatures. As this is the first attempt at the response of OV (phase I) anticancer agents in the TCGA consortium, the suggested GTI-prediction with a given signature of samples will be a valuable resource for related research projects, including a drug-response interaction analysis and bioinformatic studies in the biomedical field.

Using relationships between genetic polymorphisms and transcriptional profiles, we successfully demonstrated a novel concept for predicting individual responses to anticancer agents. Our approach might be a valuable frame work for interactome studies on drug responses and survival outcome predictions.

MATERIALS AND METHODS

Cancer samples and experimental design

A total of 249 OV (serous cystadenocarcinoma) samples were enrolled in the TCGA consortium (http://cancergenome.nih.gov) phase I trial, and they were utilized for this study. Among them, 100 Caucasian OV samples with advanced cancer stage ($\geq$ stage III) were tested for the criteria of being treated with both platinum and taxane agents, and no more than three agents in total. All samples were treated with platinum agents as the first-line anticancer agent. Survival times of selected OV samples were calculated from the date of diagnosis to the date of death or the latest follow-up to present the individual response to the anticancer drug. Finally, 99 samples passed the criteria and were used in the study (one case was discarded due to missing survival time records). The 99 samples were randomly divided into two groups: 30 samples in a "trial set" to establish the prediction model with inter-relational analysis and predictability validation, and the remaining 69 samples to validate the prediction advantage as an independent "test set". The prediction model was created with a regression approach. To determine random sampling eligibility, non-significant differences in survival times between the "trial set" (n = 30) and independent "test sets" (n = 69) were confirmed statistically (P-value from Wilcoxon rank sum test; 0.5). Additionally, we also prepared an "excluded set" of Caucasian OV samples (n = 6) that underwent a platinum plus non-taxane treatment to specify the predictions for the survival response of selected anticancer agents with OV samples in the TCGA consortium.

Stochastic analysis for the genetic polymorphisms and transcriptional profiles inter-relationships

At the time of primary surgery, tumor material was excised prior to administering anticancer agents, and genomic DNA and RNA were extracted according to the protocol of the Biospecimen Core Resource, a component of the TCGA project. The Affymetrix Whole-Genome Wide Human SNP 6.0 (WG-SNP6.0) array was used to genotype the prepared OV samples. The Affymetrix Human Genome-U133 (HG-U133) array was used for gene expression profiling of OV tumor tissues. Array hybridization was performed according to the manufacturer's protocol (Affymetrix, Santa Clara, CA, USA). As additional adjuvant therapies were heterogeneous, genes and corresponding genetic polymorphisms in the taxane and platinum related pathways were analyzed to identify the predictable factors related with the chemoresponses. To do this, we prepared member genes using the Pharmacogenomics bioinformatic database, PharmGKB (www.pharmgkb.org). The identification of promising indicative genetic polymorphisms for the drug effect, such as SNPs and haplotypes, was completed with the source of R package (http://r-project.org), SNPassoc, and haplotype analysis method (22) with an FDR adjustment of 0.05 using the R package. The indicative transcriptional signatures were identified and inter- genetic polymorphism relationships were completed with our developed Python pipe-line (www.python.org), according to the methods of Huang et al (12).

Prediction with a focus on the relationships between genetic polymorphisms and transcriptional profiles and validation

We prepared a set of candidate markers that were strictly indicative markers. All of selected genetic polymorphisms (SNPs) and transcriptional profiles were significantly associated with survival times (FDR $<$ 0.05), and their relationships were significant (FDR $<$ 0.05). The value of the inter-relationship (I) between genetic polymorphisms (Gv) and transcriptional signatures (Ts) was determined by $I = Gv\ Ts$. Survival times (S) of each OV sample after the administration of anticancer agents were predicted by the model $S \sim \Sigma Gv + \Sigma Ts + \Sigma I$. An overview of our prediction model and analysis process is depicted in Fig. 1. We called our prediction results "genetic polymorphisms, transcriptional profiles and inter-relationship (GTI)-prediction". We built other prediction scenarios to validate the advantage of using genetic polymorphisms, transcriptional signatures, and inter-relationship values. Various scenarios for predicting the outcomes from anticancer agent treatment have been reported, such as a prediction with Ts ($S \sim \Sigma Ts$), Gv ($S \sim \Sigma Gv$) without an inter-relationship value ($S \sim \Sigma Ts + \Sigma Gv$); thus, the prediction results were compared to show the improvement in the GTI-prediction.

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