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A transcription/translation-based gene signature predicts resistance to chemotherapy in breast cancer

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ABSTRACT

Although chemotherapy is widely used to treat human cancers, most chemotherapeutic agents only benefit a small fraction of patients because of the heterogeneity of cancers. Therefore, identifying of the sensitivity of cancers toward various chemotherapies would be important for choosing of chemotherapeutic regime. In this study, a 23-gene chemoresistance signature was developed from chemoresistant breast cancers. Functions of the genes in the signature were related with transcription and translation. The signature was indicative of chemoresistance and associated with poor prognosis in multiple chemotherapeutic agents and cancer types. Furthermore, by applying computational approaches, we identified several compounds that might specifically affect the chemoresistant signature. Decitabine (DAC) was the compound most likely to target the signature. In vitro and clinical analysis confirmed effect of DAC toward both breast cancer cell lines and ovarian cancers respectively. In conclusion, our study identified a chemoresistant signature that is both predictive and prognostic, and the signature-related chemoresistance could be suppressed by DAC treatment.

1. Introduction

Within the past decade, DNA microarrays have provided a new choice in the development of molecular diagnosis and prognosis of breast cancer by generating gene expression-based signatures such as the 70-gene metastasis prognostic signature MammaPrint [1] and the 21-gene recurrence signature Oncotype DX [2]. MammaPrint, accompanied with the MINDACT trial is a powerful ways to predict metastasis and chemoresponse in breast cancers with negative or 1–3 positive nodes, and it performs more precisely than traditional means of diagnosis.

At the same time, although more chemotherapeutic agents are now available and widely used to treat breast cancers, only about half of patients benefit [3], and the survival rate has not substantially improved for patients who develop chemoresistance. Traditionally, the lymph node status, tumor size, histological type, histological grade, and hormone receptor status are used to predict the response to chemotherapy, but tumors at the same stage or histopathological classification often show different responses and outcomes to a specific therapy. Therefore, it is promising and important to develop chemoresistance signatures by understanding and identifying how tumors evolve on the molecular level to overcome the various chemotherapies. Previously, several studies have identified gene-expression chemoresistance signatures, in particular to a specific chemotherapy regime in a specific subtype of breast cancer [4,5]. However, no matter whether their chemoresistance is acquired or intrinsic, different tumors may use a common mechanism in response to a variety of structurally and functionally distinct agents [6]. Therefore, this study was designed to search for a gene signature that predicts the response of breast tumors, as well as other types of tumor, to commonly used agents. To achieve this, genes whose function are associated with gene transcription and translation were selected into a chemoresistance signature, since these genes may regulate the expression of a greater range of genes and signaling pathways during the development of chemoresistance, rather than genes regulate specific processes such as apoptosis, angiogenesis and so on. As a result, it is possible that more and different signaling pathways could be included in one signature, so that the signature is more likely to be accurate to predict chemoresponse. On the basis of the signature, a randomized clinical breast cancer trial was used as the training data set to generate the signature and then validated with

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seven external data sets from either breast cancer or other types of cancer.

2. Materials and methods

2.1. Statistical analysis

Raw data of the Affymetrix microarray were downloaded from the NCBI website. The raw data were consolidated and GO-annotated in the Linux system with Perl programming language.

Cox proportional hazard model (backward stepwise selection procedure (Wald)), area under the receiver operating characteristic curve (AUC), and Kaplan–Meier were performed using SPSS (version 21) or Matlab software as previously suggested [7]. Meta-analysis was performed using Review Manager software. Hierarchical clustering was performed in Cluster 3 software.

2.2. Discriminative model

The Bayesian discriminative method using a leave-one-out cross validation was used to estimate the classifier performance of the signature [1,8,9]. Under this method, a multivariate probabilistic equation was then generated based on the mRNA level of genes in the signature, where each gene was distributed with a coefficient. By applying the equation, every patient was given a signature score. The mean value of the scores from all the patients in each cohort was used as a threshold value to discriminate chemosensitive and resistant individual.

2.3. RNA-seq and statistical analysis

The total RNA of MCF-7 cells was extracted and the mRNA was enriched using oligo (dT) magnetic beads. mRNA was fragmented into short fragments (~200 bp) and cDNA was synthesized using random hexamer-primer. DNA polymerase I was added to synthesize the second strand. End repair and 3′-end single nucleotide A (adenine) was added, ligated to sequencing adaptors, and enriched by PCR amplification. The Agilent2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR System were used to qualify and quantify the sample library. The library products were prepared for sequencing with Illumina HiSeq2000.

2.4. Cell culture, drug treatment, and MTT

MCF-7/WT cells (ATCC), adriamycin (ADM)-, and paclitaxel (PTX)-resistant human breast cancer cells (MCF-7/ADM and MCF-7/PTX) were derived as previously described [10] and cultured in RPMI.

After the MCF-7/ADM and MCF-7/PTX cells were seeded onto 96-well plates, they were treated with 5 μM DAC overnight, then serially diluted ADM or PTX was added and the cells were cultured for 48 h. Then, the cell viability was calculated using an MTT kit (Roche Applied Science, USA). IC_{50} was calculated by non-linear regression.

2.5. Real-time PCR

mRNA expression of the genes in the signature was analyzed by real-time PCR. RNAs were reverse-transcribed by Superscript II reverse transcriptase (Invitrogen). Real-time PCRs were performed using IQTM SYBR Green Supermix (Bio-Rad) for 40 cycles of 95 °C for 15 s and 60 °C for 40 s. Because the number of genes analyzed by real-time PCR is large, so the primer sequences for the 23 genes are omitted for clarity but available in supplemental Table 3.

3. Results

3.1. Development of chemoresistance signature in the discovery cohort

The gene expression data of Hatzis et al. [11] (GSE25055) from 310 breast cancer patients were used as the discovery cohort to develop a chemoresistance signature. The patients were classified as chemosensitive when they showed a pathologically complete response (pCR) to taxane-anthracycline-based chemotherapy, and patients with an extensive residual cancer burden (RCB-III) were considered to be chemoresistant; 136 of the 310 patients were then selected. The gene expression profiles of chemoresistant and chemosensitive tumors were annotated by gene ontology (GO) and compared, then a 23-gene chemoresistance signature was generated by selecting the most frequently changed transcription- and translation-related genes (Fig. 1A and Table S1). Due to the large heterogeneity of tumors between individuals, not all of the genes in the list differed significantly between the chemoresistant and chemosensitive groups, but this did not rule out their ability to predict chemoresistance as demonstrated by our subsequent studies.

3.2. Detection of chemoresistance signature in the discovery cohort

To test the performance of the signature, a Bayesian discriminative method was trained in GSE25055 to generate a multivariate probabilistic equation with as following:

$$\text{Score}_n = -28.053 + \text{TFAP4}_n + \text{EIF2AK2}_n + \cdots + \text{RPS19}_n$$

The score could be calculated for the nth patient with its mRNA level for the 23 genes in the signature. The coefficients for each gene are omitted for clarity but showed in Table S2.

A threshold value that quantitatively assessed the chemoresistant phenotype was calculated by the mean of all the scores of patients in a cohort. Patients with scores higher than the threshold were defined as chemoresistant, and vice versa. Because the difference between the types of microarray chips, as well as great heterogeneity between different groups of patients, the threshold changes between different datasets that was used in the following studies.

The accuracy of the signature was calculated as the number of patients correctly classified/total number of resistant or sensitive patients. As a result, this signature showed an accuracy of 88% and 81% for detecting chemosensitive and resistant patients, respectively (Fig. 2A and Table S2). The signature performed well in the discrimination of the chemoresponse as it showed an area under the receiver operating characteristic curve (AUC) of 0.837 (Fig. 2B).

We then estimated the chemoresponse of all 310 patients in the data set. The patients were then grouped into chemosensitive and resistant to predict the distance relapse-free survival (DRFS) by Kaplan–Meier analysis; a significantly worse DRFS rate was found in the chemoresistant patients (Fig. 2C).

Finally, using a multivariate Cox proportional hazards model, we found that the estrogen receptor (ER) status and our signature were two covariates with independent prognostic value for DRFS (Table 1), the hazard ratio (HR) of the signature to DRFS was 0.066, indicating the signature is significantly unfavorable toward DRFS. Similarly, ER status with HR of 6.735, indicating ER positive status is a favorable factor toward DRFS. Therefore, to rule out the effect of ER status on chemoresistance, we separated the patients into ER-positive and -negative (ER+ and ER−) subgroups, our signature further showed better predictive ability for DRFS in ER− than ER+ patients (Fig. 2D a and b).