

Chemogenomics approach to understanding drug-transporter interactions

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Abstract. We have applied two approaches to understanding the role of membrane transporters in drug action. First, we correlate gene expression with cytotoxic drug potencies, leading to the identification of drug-transporter interactions relevant to cancer chemotherapy. Second, we search for functional polymorphisms in transporter genes, yielding new insight into genetic causes of interindividual variability of drug response. (www.actabiomedica.it)

Key words: Transporter genes, pharmacogenomics, structure-activity relationships, genetic polymorphisms

Introduction

Membrane transporters play a key role in drug targeting or represent direct drug targets per se. However, only a few of the >400 genes encoding solute carriers (SLCs) and ABC export pumps have been studied in detail. We have developed a genomic approach for identifying transporter substrates with cytotoxic properties as candidate anticancer drugs. Similarly, the genetic causes of phenotypic interindividual differences in transporter function are poorly understood, requiring high-throughput methods for discovery.

Material and methods

For identifying novel drug-transporter interactions, we perform correlation analysis between gene expression profiles and cytotoxic drug potencies across a panel of 60 cancer cell lines used for drug discovery (NCI60). Significant correlations suggest a role in chemoresistance (negative coefficients) or -sensitivity (positive), and are then experimentally validated (1). For detection of functional polymorphisms, we have

developed rapid assays capable of measuring aberrant mRNA expression and processing. Differences in allelic mRNA expression, termed allelic expression imbalance (AEI), reveals the presence of cis-acting factors modulating transcription and mRNA processing, potentially the main causes of human phenotypic variability (2, 3).

Outcomes

Correlation analysis between gene expression and cytotoxic drug potency in the NCI60 has revealed numerous transporter-substrate interactions relevant to cancer treatments (1). Several of these correlations have been experimentally validated for select transporters (e.g., ABCB1 and 5, SLC7A11), providing insight into mechanisms of chemoresistance mediated by transporters, and structure-activity relationships for transporter substrates (4, 5). These results are relevant to the understanding of chemoresistance mediated by ABC transporters, and glutathione-dependent resistance related to the activity of SLC7A11 (cystine-glutamate exchanger).

To detect cis-acting polymorphisms, we have applied AEI analysis to >60 genes, including several membrane transporters (3,6,7). AEI then serves as a quantitative phenotype for testing linkage to measured polymorphisms in the respective gene loci. Comparison of the allelic ratios in genomic DNA with those in mRNA (after conversion to cDNA) integrates all factors determining the nature and amount of mRNA alleles present. We have applied this approach to the analysis of genetic variability in the MDR1 gene locus (ABCB1, encoding multiple-drug resistance polypeptide 1). Analysis of AEI in human liver autopsy samples, and subsequent in vitro studies, demonstrated that the synonymous SNP, C3435T, accounts for 1.5-2 fold changes in mRNA levels, associated with increased mRNA turnover (6). Applied to the serotonin transporter expressed in pontine regions of human brain autopsy tissues, AEI analysis failed to confirm a promoter repeat (SERT-LPR) as the functional polymorphisms accounting for altered gene expression (7).

Conclusions

Gene expression-drug potency correlations have proven powerful in studying drug-transporter interactions, revealing potential mechanisms of chemoresistance and -sensitivity in cancer chemotherapy. Similarly, the use of rapid AEI analyses in relevant target tissues represents powerful means of searching for functional polymorphisms, even in genes that had previously been extensively studied. Genetic variations can alter protein sequence and function, substrate recognition, and gene regulation, mRNA processing, and translation (Figure 1). Genomic surveys indicate that polymorphisms affecting transcription and mRNA processing, including splicing and turnover, may account for a main share of genetic factors in human phenotypic variability; yet, a majority of these polymorphisms likely remains to be discovered. We are systematically scanning a large number of drug targets for functional polymorphisms, revealing the presence of novel and frequent functional variants of possible pharmacological significance.

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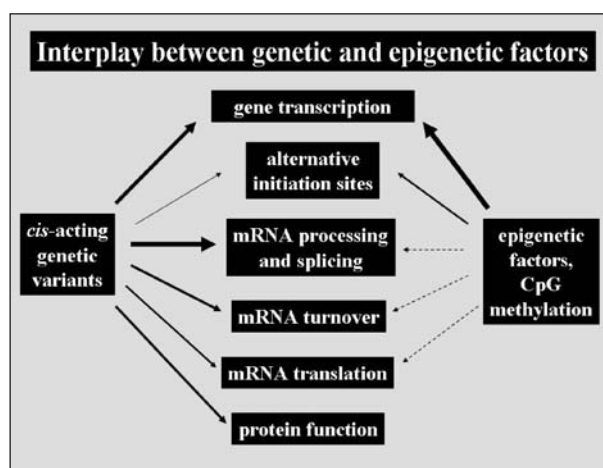


Figure 1. Schematics of the effects of cis-acting polymorphisms (see also (8)). Multiple mechanisms affect gene transcription and mRNA processing, which may be more prominent than polymorphisms directly affecting protein sequence (e.g., non-synonymous SNPs). A rough and subjective estimate of the predominance of each process is represented by the thickness of the arrow, gleaned from literature surveys. Epigenetic processes can also affect gene expression – and can be allele-specific (imprinting, X-inactivation, etc). cis-Acting polymorphisms are those located in the gene locus under study, as opposed to trans-acting factors such as transcription factors. The presence of AEI implies the presence of cis-acting factors in a given individual. Not included are regulatory processes involving noncoding RNAs, such as siRNAs and microRNAs.

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