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Systematic drug safety evaluation based on public genomic expression (Connectivity Map) data: Myocardial and infectious adverse reactions as application cases

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ABSTRACT

Adverse drug reaction (ADR) is of great importance to both regulatory agencies and the pharmaceutical industry. Various techniques, such as quantitative structure–activity relationship (QSAR) and animal toxicology, are widely used to identify potential risks during the preclinical stage of drug development. Despite these efforts, drugs with safety liabilities can still pass through safety checkpoints and enter the market. This situation raises the concern that conventional chemical structure analysis and phenotypic screening are not sufficient to avoid all clinical adverse events. Genomic expression data following *in vitro* drug treatments characterize drug actions and thus have become widely used in drug repositioning. In the present study, we explored prediction of ADRs based on the drug-induced gene-expression profiles from cultured human cells in the Connectivity Map (CMap) database. The results showed that drugs inducing comparable ADRs generally lead to similar CMap expression profiles. Based on such ADR-gene expression association, we established prediction models for various ADRs, including severe myocardial and infectious events. Drugs with FDA boxed warnings of safety liability were effectively identified. We therefore suggest that drug-induced gene expression change, in combination with effective computational methods, may provide a new dimension of information to facilitate systematic drug safety evaluation.

1. Introduction

Adverse drug reactions (ADRs), also referred to as side effects, are a major public health concern. The number of deaths caused by ADRs is estimated to be comparable to that from common diseases, such as cancers and cardiovascular disorders [1]. ADRs also lead to failures of drug development and drug withdrawals from the market, thus elevating the overall cost of drug development and reducing the number of newly approved drugs [2].

Many safety assessment methods, such as quantitative structure–activity relationship (QSAR) modeling [3], preclinical animal tests, and phase 1 clinical studies in humans, are developed to set checkpoints at early stages of the drug development pipeline. However, some drugs with safety risks still enter the market, resulting in severe injuries and even loss of lives [4]. This situation suggests that conventional methods based on chemical structure [5,6] and human/animal phenotypes are necessary but not sufficient to detect all the various kinds of human safety risks [7], especially some latent reactions that are detectable only after long-term clinical use [8]. A series of novel computational models have therefore been established as alternative solutions to early prediction of ADRs. These models are based on various types of large-scale information, such as pharmacological networks [9,10], biochemical assays [11] and systematic chemical characteristics [12–14], but relatively less progress has been made regarding high throughput gene-expression data [15].

CMap is a transcriptomic data collection developed from 6100 human cell cultures treated with 1309 bioactive compounds and matched vehicle controls. For each compound, the expression changes of 22283 gene probes collectively constitute an expression profile. For each profile, a small set of gene probes with the highest fold change compared to vehicle controls were used as drug-specific signatures [16]. One potential limitation of the use of this vast

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transcriptomic dataset is the so-called batch effect, in which gene expression changes due to a drug may be confounded by cell culture conditions and other uncontrolled variables [17,18]. Since cell cultures treated with the same compound but in different batches should differ more in batch variation and less in drug action, we used the expression profiles of these cultures to estimate and normalize batch variation. The signatures of normalized expression profiles were compared to each other using the Gene Set Enrichment Analysis (GSEA) algorithm [19], so the similarity between different drugs could be measured by Bridge Adjusted Expression Similarity (BAES) [20].

Since high similarity scores may reveal common actions of different drugs, CMap has been widely used to predict new drug indications (i.e., drug repositioning) [21–24]. But much less attention being given to the potential association between CMap and drug side effects [25]. Here we found drug-specific gene expression profiles as a guilt-by-association indicator of ADRs and established a 'risk score' model to computationally predict drug safety risks. The results showed the unique advantages of this CMap-based model, which included the prediction of ADRs that are difficult to detect with traditional methods.

2. Materials and methods

2.1. Selection of reference drugs according to boxed warning information

The CMap drugs subjected to records in DailyMed up to March 2014 were manually collected from the official website at http://dailymed.nlm.nih.gov/. And the box warning information was independently validated by 2 experts to determine the list of reference drugs. To avoid subjective bias of selection and keep only the most straightforward information, only the boxed warnings suggesting direct associations between one drug and one side effect were used to select reference drugs. Other warning information was strictly screened out (Table S1), such as concomitant administration of multiple drugs, exacerbation of preexisted conditions, consequence of discontinuance, etc. The ADRs with similar symptoms or correlated toxicology were pooled together as an ADR class (Table S2). For instance, 'acute liver failure' and 'hepatic necrosis' were both categorized as drug-induced liver injury (DILI). A total of 25 ADR classes were associated to at least one CMap drug (Data S1). To ensure statistical power, only those ADR classes linked to at least 10 drugs were addressed with risk score model.

2.2. The assessment model of risk score

The risk score of a certain drug was measured by its overall gene-expression similarity to a series of reference drugs with boxed warnings. If N ($N > 0$) reference drugs are used in the model, then the risk score of a test drug is estimated with this formula

$$Risk = \frac{\sum_{i=1}^{N} S_i}{N},$$

where S_i is the expression similarity, in terms of BAES score, between the test drug and the i-th reference drug.

3. Results

3.1. Drug side effects are characterized by genomic expression profiles

We primarily retrieved drug label information from the DailyMed database (http://dailymed.nlm.nih.gov/) [26]. The highest level of safety risks or adverse effects are usually suggested by the boxed warning (also referred to as 'black box warning'), which is the strongest warning that the FDA requires on drug labels. On the other hand, the risks of less concern are mostly indicated in the 'warnings and precautions' section. In the present study, all CMap drugs were queried in DailyMed for boxed warning information. A total of 293 drug-ADR relationships between all CMap drugs and 25 major ADR classes were identified as 'gold standard' (Data S1). Our basic hypothesis is that drugs showing high expression similarity were prone to induce similar side effects. Therefore, the drugs warned for direct association with specific clinical adverse events were used as 'reference drugs' (see Methods). Then the risk of a new test drug was estimated according to its expression similarity to reference drugs, i.e. the higher the similarity, the higher the likelihood of safety liability it may suggest (Fig. S1).

To verify our hypothesis, we primarily confirmed that with reduced batch variation in CMap data, drugs warned for the same class of ADRs showed generally higher expression similarity (in terms of BAES score) than random drugs (Fig. 1A–D). Moreover, we noticed that drugs associated to the same ADR class were enriched in the drug pairs with the highest similarity. The ADR-expression association suggested that not only therapeutic effects, but also side effects can be characterized by transcriptomic profiles. In particular, a variety of severe ADRs affecting different organs and tissues were highly associated with characteristic expression patterns (Fig. 1E). As a result, we developed computational models to detect potential drug safety risks, including drug-induced myocardial reactions and serious infections.

3.2. Case study: drug-induced myocardial adverse reactions

Myocardial toxicity is one type of life-threatening adverse reaction, and also one of the most common causes of drug withdrawals from worldwide markets [27]. Because of the unexpected myocardial reactions associated with the wide-scale use of Vioxx® (generic name rofecoxib) [28], damage to the heart muscle (e.g., myocardial infarction and heart failure) has become a major focus of drug safety. However, the toxicological mechanisms of myocardial adverse reactions remain poorly understood, which makes prospective safety assessment difficult.

Here we retrieved from DailyMed a set of reference drugs associated with adverse myocardial reactions, such as myocardial infarction, heart failure and congestive heart failure etc. Since drugs with comparable ADRs tend to produce correlated expression profiles in CMap, the likelihood of myocardial ADRs of a specific drug may be quantitatively characterized by its overall expression similarity (i.e., BAES score) to those of reference drugs warned for myocardial risks; the higher the similarity, the greater the odds of that specific drug causing ADRs (Fig. 2A). To verify this theory, we adopted a previously published naïve model [20] that equally weights expression similarity to individual reference drugs and translates ADR risk into a 'risk score' (see Methods).

The performance of risk score model was evaluated by leave-one-out cross validation (LOOCV) and visualized by receiver operating characteristic (ROC) curve. With reference and non-reference drugs all ranked in the order of risk score value, the efficiency of detecting reference drugs was quantified by the indexes of sensitivity and specificity (Fig. 2B). Setting the 98% quantile of risk score of non-reference drugs as the threshold (i.e., under the premise of 98% classification specificity), we identified 25% reference drugs above the threshold. That means drugs with boxed warning are 12.5 times more likely to be found among those with the highest risk scores than random inspection (Fisher's exact $p = 5.9 \times 10^{-7}$), suggesting the solid characteristics of warned drugs.

Given the guidance of risk score, we paid attention to not only the reference drugs but also the non-reference ones subjected to

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