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Exploration of individuality in drug metabolism by high-throughput metabolomics: the fast line for personalized medicine

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In many cases, individuality in metabolism of a drug is a reliable predictor of the drug efficacy/safety. Modern high-throughput metabolomics is an ideal instrument to track drug metabolism in an individual after treatment. Productivity and low cost of the metabolomics are sufficient to analyse a large cohort of patients to explore individual variations in drug metabolism and to discover drug metabolic biomarkers indicative of drug efficacy/safety. The only potential disadvantage of metabolomics becoming a routine clinical procedure is a need to treat the patient once before making a prognosis. However, in many clinical applications this would not be a limitation. Here, we explore current opportunities and challenges for translating high-throughput metabolomics into the platform for personalized medicine.

Introduction

Q2 Personalized medicine is a very active area of research with enormous potential [1]. The central target of personalized medicine is the development of accurate diagnostic tests that are capable of recognising the most beneficial therapy for a patient. The fast development of high-throughput technologies enables systematic profiling of biological systems at the basic level. Current technologies enable interrogation of patient individuality on a molecular level to match the optimal treatment [2]. There are multiple examples that provide excellent demonstrations of the power of new technologies to deliver a range of biomarkers. Most efforts in this area are currently focused on predictive genetic tests using genotyping or HTS technology [3,4], whereas the potential of other omics platforms has been underestimated.

The individual differences in the ability to metabolise and detoxify drugs have been long since recognised in toxicology [5]. Variability in drug metabolism is commonly associated with polymorphism in drug-metabolising enzymes, in particular the cytochrome P450s (CYPs). There are multiple examples of drug toxicities that can be predicted based on CYP polymorphism [6,7]. Recently, individuality in drug metabolism was recognised as an important factor in drug prescribing practices, in particular in oncology [8]. For example, tamoxifen is metabolised by CYP2D6 and transformed into biologically active metabolites (4-OH tamoxifen and endoxifen). Polymorphism of CYP2D6 could be a reason for inefficient transformation of tamoxifen into active products and, thus, is likely to be indicative of poor response to the drug [9]. The CYPs are not the only enzymes that can be involved. For example, irinotecan, a standard treatment for advanced colorectal cancer, is metabolised through a complex pathway involving glucuronosyl transferase, an enzyme encoded by UGT1A1. Commercial tests have been developed and approved for detecting the UGT1A1 genotype in connection with irinotecan therapy [8,10,11]. These examples demonstrate that individuality in metabolism and pharmacokinetics of a drug is actively exploited as a biomarker to personalize treatment. Q3

Drug metabolism is a complicated process involving multiple enzymes [12]. Most drugs are actively metabolised by the total cellular enzymatic machine to produce dozens of metabolites [13–15], some of which might be specific for patient subgroups [9]. In addition to being bioactive and indispensable from primary

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drug action mechanisms, other metabolites could be responsible for adverse side effects [5]. Variability of drug metabolism is controlled by patient genetics. However, availability of the patient genotype or genome would not automatically lead to the understanding of all metabolic roots for a given drug. Deciphering the role of genetic polymorphisms in the metabolism of a particular drug is a challenge that is far from being solved. Any single genetic test would, in principle, be limited to a particular mechanism reflected by the tested genotype, whereas the remaining profile of drug metabolism in an individual would remain hidden.

Recent progress in high-throughput metabolomics offers unprecedented possibilities for a systematic study of various aspects of cell metabolism [16–18]. High-throughput metabolomics has been widely used as a powerful instrument to study drug metabolism and, at the moment, is applied mostly at the early development stages of drug discovery to understand primary mechanisms of drug clearance, drug pharmacokinetics and pharmacodynamics [14,15,19]. However, application of metabolomics to understand variability of drug metabolism among individuals on a subpopulation scale has been rare. Also, metabolomics has not been considered as a clinical tool to monitor drug metabolism in patients with the purpose of making early predictions (after the first treatment) of long-term drug efficacy/safety. Low cost and throughput of the current metabolomics platforms are sufficient to analyse a large cohort of patients [9,16,20]. Technically, there are no principal limitations to organise large-scale clinical studies to track variations in drug metabolism for approved drugs (or drugs in final stages of clinical trials) and to link them with variations in drug clinical outcomes. As a result, biomarkers (drug metabolic products) that are indicative of the drug efficacy/safety could be discovered. The only principal disadvantage of metabolomics as a platform for personalized medicine to track individuality in drug metabolism is the need to treat a patient once before the prognosis could be made. However, in many clinical applications this would not be a limitation. For example, in oncology the patient response to a drug could be determined reliably after just weeks of treatment because most drugs are toxic [21]. Thus, reliable prediction of the drug efficiency/safety after one treatment would be of paramount importance. Here, we explore challenges and opportunities of high-throughput metabolomics on the way to becoming a powerful platform for personalized medicine.

High-throughput metabolomics: current viewpoint

The most recent advance in high-throughput metabolomics is mainly associated with resolution improvement of mass spectrometry (MS), namely with introduction of Fourier transform (FT), time-of-flight (TOF) and Orbitrap instruments [16,17,22]. The accuracy of these platforms enables mass determination beyond 1 ppm (i.e. with precision of ~0.001 Da for most metabolites). This makes it possible to resolve several-thousand metabolites in a single spectrum without the need for chromatography [16,20,23]. The typical mass spectrum of a biological sample would have from 10,000 to 30,000 mass peaks. Metabolite candidates can often be assigned to a mass peak with reliable confidence based on accurate mass alone [16,17,24–26]. Fragmentation of a mass peak (MS/MS) in most cases would fully resolve the metabolite identity and can even help to differentiate between mass isomers. These experimental technical capabilities move the bottleneck of metabolism research from experimental data generation to computational challenges in data interpretation.

The common approach dealing with high-throughput metabolomics data is to match mass peaks by querying cell metabolism databases [27,28] (Fig. 1a). Omitting some technical aspects, in this case an almost complete list of known metabolites (covered by the corresponding database) present in the sample would be identified. The other approaches can discover novel metabolites not yet known, like specific lipids, but require fragmentation of a mass peak [29]. The fact that metabolites in the biological sample are educts and products of each other makes it possible to match mass peaks that represent a metabolic reaction (a pair of metabolites that are transformed into each other) (Fig. 1b). This can be exploited to infer from MS data not only a list of metabolites present in the sample but also a list of metabolic reactions (Fig. 1c) that are active in the sample [28,30].

High-throughput metabolomics: drug metabolism

MS-based high-throughput metabolomics has been commonly applied to understand the major roots of drug metabolism at early stages in the drug discovery process [19]. Most of the drug metabolic products in a biological sample (e.g. blood plasma, bile, urine) would be detected even at reasonably low concentrations by modern MS platforms. The main challenge is computational: to decipher peaks in a mass spectrum that correspond to the drug metabolic products (several dozen) from peaks corresponding to other natural metabolites or artefacts (several thousands). Information on the metabolic roots of most drugs is usually very sparse. This stimulated the development of computational tools to predict potential metabolites from the drug chemical structure. To date, several procedures have been used.

Mass defect filtering (MDF) is a data-mining technique specifically developed to predict drug metabolites from full MS data generated by high-throughput metabolomics [31,32]. MDF imposes a filter on the residue mass commonly within a window of 40–50 mDa relative to the parent drug mass or masses of its core substructures. The principle behind MDF is based on the idea that the atom composition of most biotransformation reactions does not exceed 2–5 atoms and, thus, mass defect shift of corresponding drug metabolites, in general, should not exceed 40–50 mDa. Another computational approach is to use knowledge-based predictions of the most probable drug metabolites. In this case, the comprehensive database of metabolism information, such as MDL® Metabolite or the Accelrys Metabolism Database has been employed. The metabolic pathways of a drug could be predicted on the basis of the knowledge-based information available for similar structures [15]. Both approaches demonstrate effectiveness for drug metabolite identification for *in vitro* and *in vivo* models [14,15].

Although the number of known metabolic reactions regularly occurring in the cell exceeds several thousand [27], the majority of these reactions can be reduced to a small list of common metabolite nonspecific biotransformations, like acetylation, carboxylation, hydroxylation or methylation. This can be used to develop a computational pipeline that will generate a network of all possible multistep biotransformations for the input molecule

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