This Month in APR

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Overview of human cytochrome P450 networks in chemical space

In the clinical setting, the discovery of the genetic polymorphism involved in debrisoquine (Mahgoub et al., 1977) and sparteine (Eichelbaum et al., 1978) metabolism and the presence of isozymes of cytochrome P450 (CYP450) (Thomas et al., 1976) and other metabolizing enzymes has emphasized the importance of individual response to drugs. The discovery of the role of CYP450 in metabolism by Omura and Sato (Omura and Sato, 1962), and Estabrook, Cooper and Rosenthal (Estabrook et al., 1963) was an invitation to scientists from many peripheral disciplines to take an interest in metabolic transformations. CYP450s constitute a superfamily of monooxygenases that is critical for both anabolic and catabolic metabolism in all organisms characterized so far. It is well known that 75% of drug metabolism is mediated by CYP enzymes; hence, CYPs play the most important role in human drug metabolism (Guengerich, 2008). The largest concentration of P450s is found in the liver, although high concentrations can also be found in intestinal and adrenal tissues. In cells, the enzymes are primarily located in the endoplasmic reticulum. Specifically, P450 enzymes are involved in the metabolism of various endogenous and exogenous chemicals including steroids, bile acids, fatty acids, eicosanoids, xenobiotics, environmental pollutants and carcinogens. The diverse endogenous function and the essential role of P450s in drug metabolism and toxicology further emphasize the importance of these enzymes in human health. In humans, there are 18 P450 families, 44 subfamilies and 57 putative functional enzymes (Nelson et al., 1996). However, the functionality, substrate specificity and tissue specificity vary among these P450 enzymes.

Despite the rapid progress that has occurred in elucidating the role of these cytochrome P450 enzymes in drug metabolism and drug-drug interactions, global analysis of the chemical space of CYP450 enzymes in humans, including the interrelationship or networks connecting them is lacking. Nearly 18 years ago, global analysis of CYPs was conducted based on sequence identity. The authors have classified the various isozymes and standardized the nomenclature using multiple step sequence alignment of the CYPs primary sequence (Nelson and Strobel, 1987; Gonzalez, 1990; Gonzalez and Gelboin, 1992; Degtyarenko and Archakov, 1993). CYPs sequences that shared >40% or >55% amino acid sequence identity are classified into the same family or the same subfamily, respectively. In a recent study, Yang et al. applied a systems biology approach via the integration of genetics, gene expression and enzyme activity measurements to measure P450 activities of 466 human liver samples. This systematic survey provided a comprehensive view of the functionality, genetic control and interactions of P450s (Yang et al., 2010). Although these studies analyze CYPs globally, the global characteristics of CYPs based on "chemical space" have not yet been determined.

One of the gaps in our understanding of the global characteristics of CYPs based on "chemical space" appears to have been clarified in this publication appearing in the current issue of *Archives of Pharmacal Research*, marking an important step in the global analysis of the chemical space of CYP450 enzymes in humans. Specifically, the authors have approached this predicament by analyzing the global characteristics and relationships between each CYP isozyme and building global CYP networks at the family, subfamily and gene levels based on three chemical space properties (P450 substrates, inducers and inhibitors) (Fig. 1). The lists of substrates, inducers and inhibitors of all known human CYPs were extracted from the integrated informational resource on CYPs. A series of six filters were employed to remove the redundancies in the dataset. The CYP-substrate network (CSN), CYP-inhibitor network (CHN) and CYP-inducer network (CDN) were built at three levels of hierarchy. For example, the three CSNs that were generated, CSN-1, CSN-2 and CSN-3, are networks for the CYP family, CYP subfamily and individual CYP genes, respectively. All of these networks were built using the CytoScape v2.6.3 software. The centrality of particular chemical space properties was projected by measuring the node degree, betweenness and closeness for each node within these networks. Additionally, promiscuity maps and heat maps were constructed based on the three chemical-space characteristics to illustrate the relationships and similarities between each CYP.

Several potent factors regarding CYPs were highlighted in this CYP network. The five major CYPs, 3A4, 2D6, 2C9, 2C19 and 1A2, which play a vital role in drug metabolism occupied more than 65% of the total number of CYP-substrate interactions and have

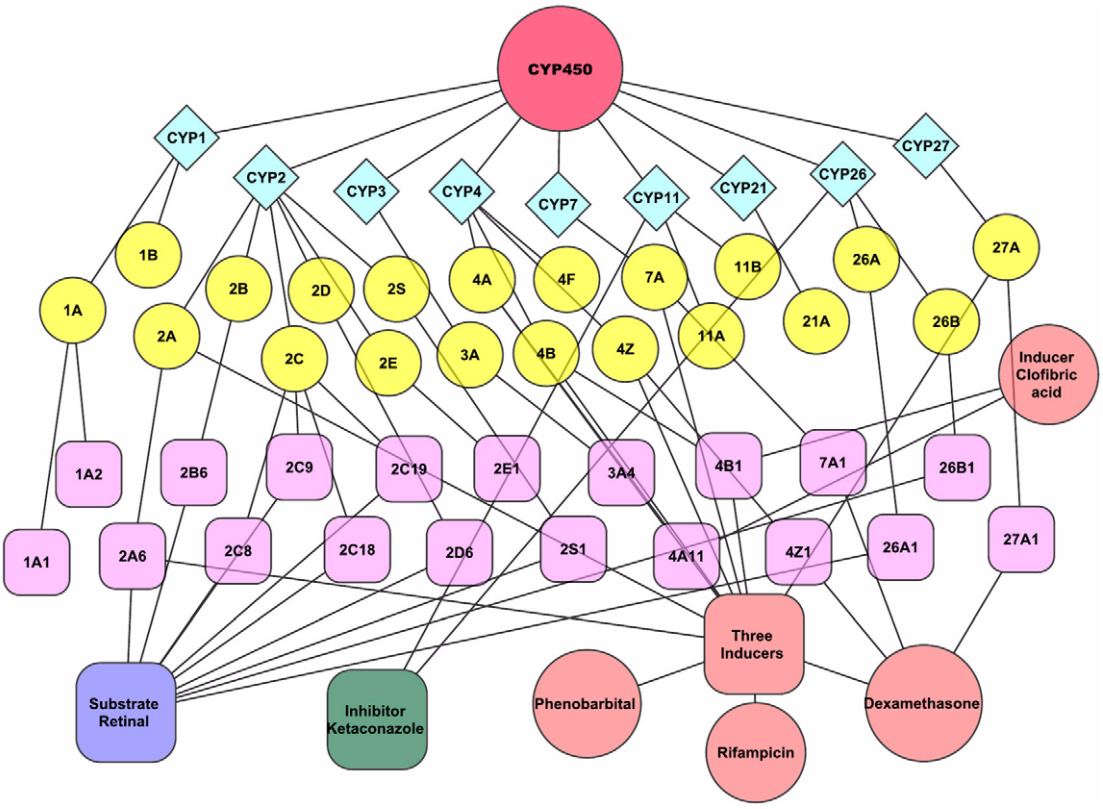

Fig. 1. Overall view of human CYP450 network at the family, subfamily and gene levels based on chemical spaces. CYP families 1-3 are responsible for 70-80% metabolism of clinically used drugs. CYP3A4 gene is the most promiscuous isozyme and is considered to be the important gene for drug metabolism. CYP2C subfamily shows high centrality in networks based on inhibitor and inducer space. CYP3A subfamily shows high degree and betweenness values in all networks, indicating very high centrality. It is important not only for drug metabolism, but is also a central family based on inhibitor and inducer space. CYP2A and 27A subfamilies show high centrality in network generated from the inhibitor and substrate space. Pale-blue color representing CYP family; Yellow color representing CYP subfamily; Light-pink color representing CYP gene. Lavender color representing common substrate retinal shared at gene level. Light orange color representing four inhibitors shared at subfamily and gene levels. Green color representing common inducer shared at subfamily level.

been listed in the top five promiscuous isozymes that have many substrates. CYP3A4 has been identified as the most promiscuous isozyme based on its relationship with both inhibitors and inducers. Additionally, substrates of CYPs 1 to 3 occupied approximately 95% of the entire substrate space. CYP2 occupied more inhibitor space than CYP3, while CYP1 occupied less inhibitor space than CYP3. CYP2 has the most substrates and inhibitors, while CYPs 3 and 1 each have more inducers than CYP2. In the CYP networks on chemical spaces, inducers were found to be more specific to CYPs than inhibitors and the specificity of substrates on CYPs falls between the specificities of inhibitors and inducers.

Promiscuity maps were designed to quantify the strength of the relationships between two nodes using the total number of connections. These maps indicated that the CYP2 family is in a hub within CSN-1 due to the large number of inter- and intra-links. This study also suggested that CYP2 may be considered a very important family based on inhibitor space. At the family level, CYP2 occupied the central position of the substrate space. Conversely, even though the sum of the number of shared substrates between all genes in the CYP2 family is greatest at the single gene level, CYP3A4 is identified as the key gene in the map. The characteristics of the promiscuity map based on inhibitors are more similar than the results based on substrates and inducers, and the pattern of promiscuity based on the inducer space is unique. Heat maps were generated to show the similarities among CYP genes based on chemical profiles. Several facts were observed in this study, such as genes in the same family do not metabolize the same substrates, genes in the same family are not grouped based on the similarities in their substrate profile, and the substrate profile of genes within the same family can be different. In this map, the relationships between CYPs and chemicals

are based on the method by which CYPs metabolize chemicals (substrates); conversely, in the heat maps of the inhibitors and inducers, the relationships between CYPs and chemicals are based on how chemicals act (inhibit or induce the activity) on CYPs.

In this paper, both intra- and inter-linking characteristics between CYP families, subfamilies and genes were observed. The relative correlations of a given cytochrome P450 isoform with other isoforms, as well as the similarity of the metabolizing ability and changes in patterns in response to chemicals were also exemplified. It also provides insight into the similarities between CYP metabolism patterns (substrates), patterns of chemical inhibition and induction of CYPs, and their cooperative properties.

The study by Lee and Kim work is noteworthy because it is the first global analysis of CYPs based on chemical-space characteristics. The authors have convincingly shown the relationships of individual CYPs with drug metabolism (substrate) and drug induced changes in activity (induction and inhibition), the degree of centrality and promiscuity in CYP networks, the correlation between pairs of CYP families, subfamilies and genes, and the informative capacity of chemical spaces on CYP metabolism and activity change. The analysis of the literature presented above allows the utilization of CYP networks in chemical space for future studies in the prediction of drug-drug interactions, phylogenetic analysis of CYPs and the research with respect to the cooperative properties of CYPs.

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