Association between the CYP11 family and six cancer types

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Abstract. Cytochromes P450 (CYPs) are a major source of variability in pharmacokinetics and drug response. CYPs utilize a variety of small and large molecules as substrates in enzymatic reactions. The CYP genes may be divided into two groups: Endogenous CYPs (CYP family 7-51) and xenobiotic CYPs (CYP family 1-4). The aim of the present study was to investigate whether endogenous CYPs exhibit similar gene expression and mutations in various cancer types. The gene expression profiles and somatic mutations exhibited in colon adenocarcinoma, kidney renal clear cell carcinoma, liver hepatocellular carcinoma, lung squamous cell carcinoma, prostate adenocarcinoma and uterine corpus endometrial carcinoma were analyzed using data obtained from The Cancer Genome Atlas. The expression of CYP11A1 was significantly down-regulated in all six cancer types. In addition, CYP11B1 and CYP11B2 exhibited the highest number of mutations among endogenous CYPs in all samples. As the CYP11 family is important for steroid biosynthesis, and previous studies have demonstrated that steroid hormones are associated with certain cancers, these results indicate a common role of the CYP11 family in various cancer types.

Introduction

Cytochromes P450 (CYPs) belong to a superfamily of proteins that contain a heme cofactor. They are primarily located in the inner membrane of mitochondria or the endoplasmic reticulum of cells (1). CYPs, as the oxidase enzymes in electron transfer chains, catalyze a number of enzymatic reactions involving small molecules. The CYPs are classified as endogenous CYPs or xenobiotic CYPs. The endogenous CYPs are involved with the biosynthesis or catabolism of steroids, sterols, retinoids, prostaglandins and fatty acids, while the xenobiotic CYPs function to defend against environmental toxins and carcinogens (2). The human CYP family 1-4 includes the major enzymes involved in drug metabolism, accounting for ~75% of the total CYPs (3).

Recently, CYP studies have increasingly focused on drug metabolism (3). However, the systematic association between endogenous CYPs and cancer remains unclear. Thus, the aim of the present study was to investigate the association between endogenous CYPs and cancer. Using data obtained from The Cancer Gene Atlas (TCGA), the gene expression profiles and somatic mutations of endogenous CYPs were analyzed in six cancer types in order to determine whether any common features may exist.

In the human body, the CYP11 gene family, including CYP11A1, CYP11B1 and CYP11B2, is one of the families of CYP genes involved in steroid biosynthesis (2). Previously, almost no research has been conducted with regards to the CYP11 family and cancer; therefore, the present study aimed to investigate the associations between them. The results of the present study may be important for expanding the global view of cancer research.

Materials and methods

TCGA. Data was obtained from TCGA (http://cancergenome.nih.gov/) (4). Data regarding six diverse cancer types, including colon adenocarcinoma (COAD), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD) and uterine corpus endometrial carcinoma (UCEC), were selected for analysis, to cover cancer types belonging to digestive system, respiratory system and reproductive system. The data set included 2,754 gene expression samples and 1,461 somatic mutation samples (Table I). The gene expression samples were further divided into two groups, which included 2,450 carcinoma and 304 pericarcinoma tissue samples.

Gene expression analysis. Endogenous CYPs were selected from the gene expression profile. The Wilcoxon signed-rank test was performed to analyze the differential expression of CYPs between carcinoma and pericarcinoma tissue for each cancer type.

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Table I. Expression and mutation of 56 genes in six cancer types.

Cancer type	Cancer samples, n	Normal samples, n	Mutated samples, n	Endogenous CYP mutations, n
COAD	433	42	154	30
KIRC	516	73	417	70
LIHC	148	51	203	25
LUSC	490	51	178	123
PRAD	334	51	261	26
UCEC	529	36	248	275
Total	2450	304	1461	549

CYP, cytochrome P450; COAD, colon adenocarcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

Somatic mutation analysis. Data regarding the somatic mutations in endogenous CYPs were obtained (Table I). Subsequently, PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) (5) was used to evaluate the severity of these mutations. PolyPhen-2 is a tool that predicts the possible impact of an amino acid substitution on the structure and function of a human protein using physical and comparative considerations. PolyPhen-2 generates a score for every mutation, which ranges between 0 and 1, whereby a higher score indicates a more severe mutation. Thus, the severity of mutations may be divided into three categories: Probably damaging, possibly damaging and benign. PyMOL (http://www.pymol.org/) was used to create the protein secondary structure. PyMOL is a molecular visualization system on an open-source foundation. The protein structure data of CYP11B2 was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB; http://www.rcsb.org/pdb/home/home.do).

Pathway analysis. The Kyoto Encyclopedia of Genes and Genomes (KEGG) PATHWAY database (6) (http://www.kegg.jp/kegg/pathway.html) was used to identify the metabolic pathway of CYPs. KEGG presents a collection of databases that contain information regarding genomes, biological pathways, diseases, drugs and chemical substances. KEGG is utilized for bioinformatics studies, including data analysis in genomics, metagenomics and metabolomics, modeling and simulation in systems biology and translational research in drug development.

Results

CYP gene expression profile in six cancer types. To identify differences in CYP expression patterns in various cancers, the expression of various genes was analyzed. The expression of CYP11A1 was significantly downregulated in all cancer types ($P<0.001$), while the expression of CYP27B1 was significantly upregulated ($P<0.001$) (Fig. 1A).

CYP11A1 is a mitochondrial enzyme that catalyzes the conversion of cholesterol to pregnenolone. This represents the first reaction in the process of steroidogenesis in all steroid hormone-producing mammalian tissues (Fig. 1B) (7). Low expression of CYP11A1 may cause steroid biosynthesis disorders (Fig. 2).

As shown in Fig. 1A, CYP27B1 was upregulated in 5/6 of the cancer types analyzed, with the exception of KIRC. CYP27B1 is most commonly identified in the proximal tubule of the kidney and a variety of other tissues, including skin, immune cells and bone. The enzyme catalyzes the hydroxylation of calcifediol to calcitriol (the bioactive form of vitamin D) (8).

Somatic mutations in the six cancer types. In the present study, the 10 most commonly mutated genes were identified and their functional effect was predicted using PolyPhen-2. The results revealed that all members of the CYP11 family, including CYP11A1, CYP11B1 and CYP11B2, were among the 10 most commonly mutated genes. In particular, CYP11B1 and CYP11B2 exhibited the most mutations among all genes analyzed. The major function of the CYP11 family is to promote steroid biosynthesis (9). CYP11B1 is a steroid hydroxylase present in the zona glomerulosa and zona fasciculate (10,11) that generates cortisol from 11-deoxycortisol and corticosterone from 11-deoxycorticosterone. CYP11B2 is a steroid hydroxylase enzyme involved in the biosynthesis of the mineralocorticoid aldosterone. The CYP11B2 protein is only expressed in the zona glomerulosa (12) of the adrenal cortex and is primarily regulated by the renin-angiotensin system (11). CYP11B2 is the only enzyme capable of synthesizing aldosterone in humans and is important for electrolyte balance and blood pressure regulation (13). CYP7A1 exhibited the second highest number of mutations among the genes studied. It also exhibited the highest number of 'probably damaging' mutations (Fig. 3A). CYP7A1 is the rate-limiting enzyme for the synthesis of bile acid from cholesterol via the classical pathway, catalyzing the formation of 7-alpha-hydroxycholesterol (14).

As the structure of CYP11B2 may be indicated in RCSB PDB, PyMOL was used to visualize somatic mutations in the structure of CYP11B2 (Fig. 3B). Approximately 80% of mutations occurred in the α-helix region. These mutations may alter the protein structure and subsequently affect its function.

Steroid biosynthesis pathway analysis. For the expression and mutation analysis of crucial members of the CYP11 family,

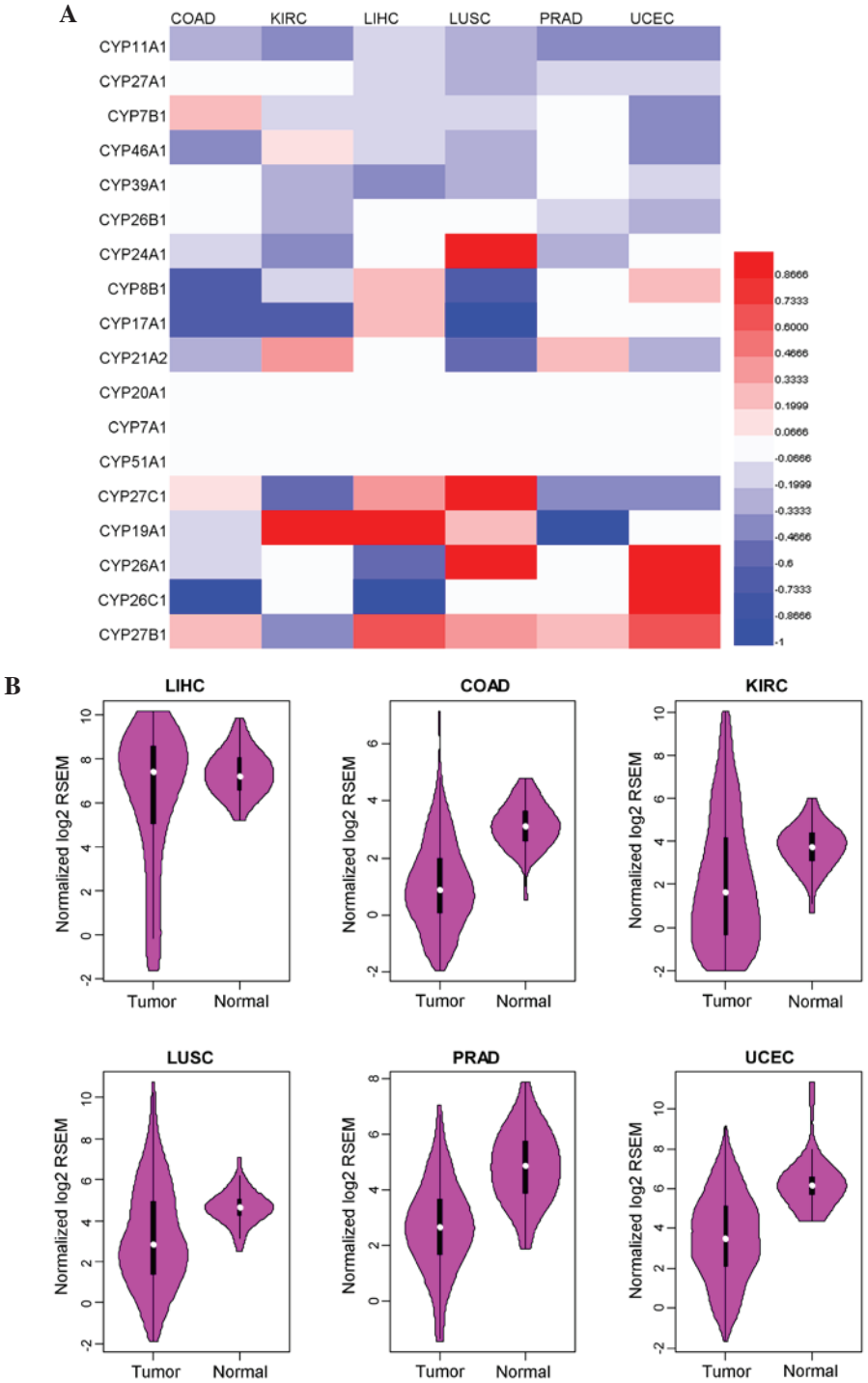

Figure 1. (A) Analysis of CYP expression in cancer and normal tissue samples showing the regulation of genes in six cancer types. A red color corresponds to upregulation and blue corresponds to downregulation. The Wilcoxon signed-rank test was used to identify significant differences in gene regulation between tumor and normal tissues. (B) Vioplot of CYP11A1 expression, showing the normalized log2 RSEM of CYP11A1 in six cancer types. CYP, cytochrome P450; COAD, colon adenocarcinoma; KIRC, kidney renal clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUSC, lung squamous cell carcinoma; PRAD, prostate adenocarcinoma; UCEC, uterine corpus endometrial carcinoma.

the KEGG pathway database was used to investigate the role of the CYP11 family in metabolic pathways. Members of the CYP11 family are involved in steroid biosynthesis. The low expression of CYP11A1 may lead to decreased expression of pregnenlone and 17α-hydroxy pregnenolone. Furthermore, mutations in CYP11B1 and CYP11B2 may affect cortisol and aldosterone levels. Pregnenolone, cortisol and aldosterone are crucial components involved in steroid biosynthesis (Fig. 2).

Pregnenolone is a type of endogenous steroid, and is the forerunner of several steroids, including glucocorticoids, mineralocorticoids, progestogens, estrogens and androgens (15). Furthermore, pregnenolone is a biologically active neurosteroid (15). Pregnenolone is synthesized from cholesterol, a transversion that requires hydroxylation at the C20 and C22 positions of the side-chain and is performed by the enzyme CYP11A1, which is located in the mitochondria

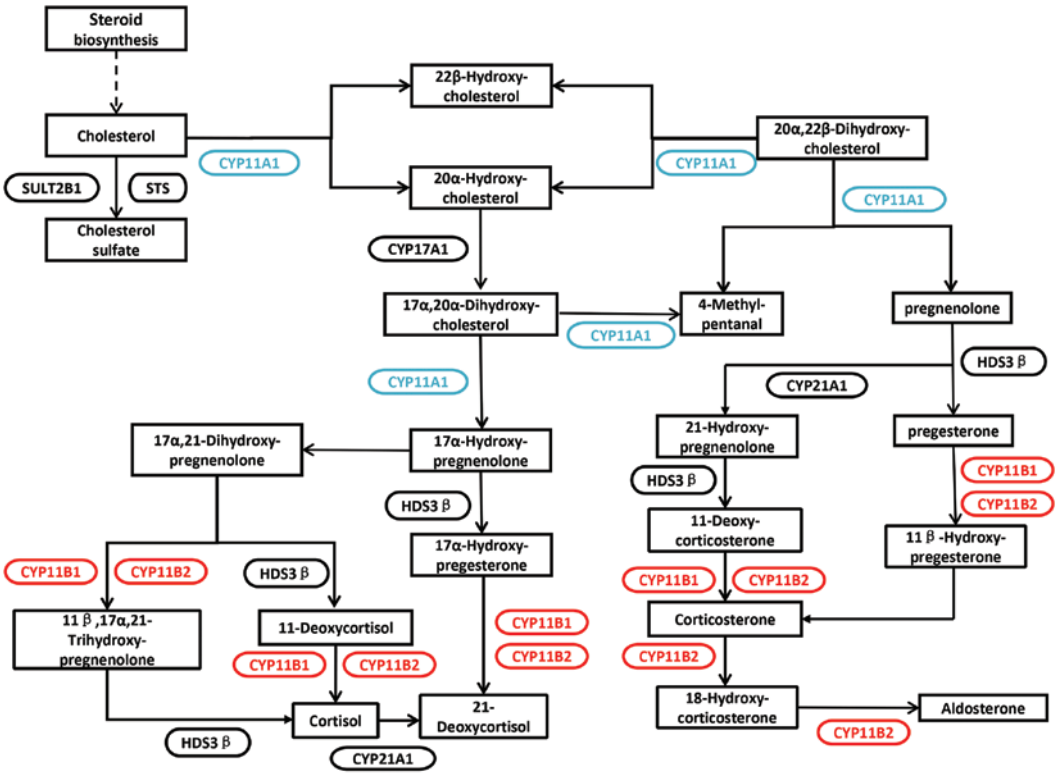

Figure 2. Steroid biosynthesis pathway. CYP11 family is involved in numerous steroid biosynthesis pathways. Blue color corresponds to downregulated expression; red color corresponds to mutation. CYP, cytochrome P450; SULT2B1, sulfotransferase family cytosolic 2B member 1; STS, sequence tagged site; HDS3β, hydroxysteroid dehydrogenase 3β.

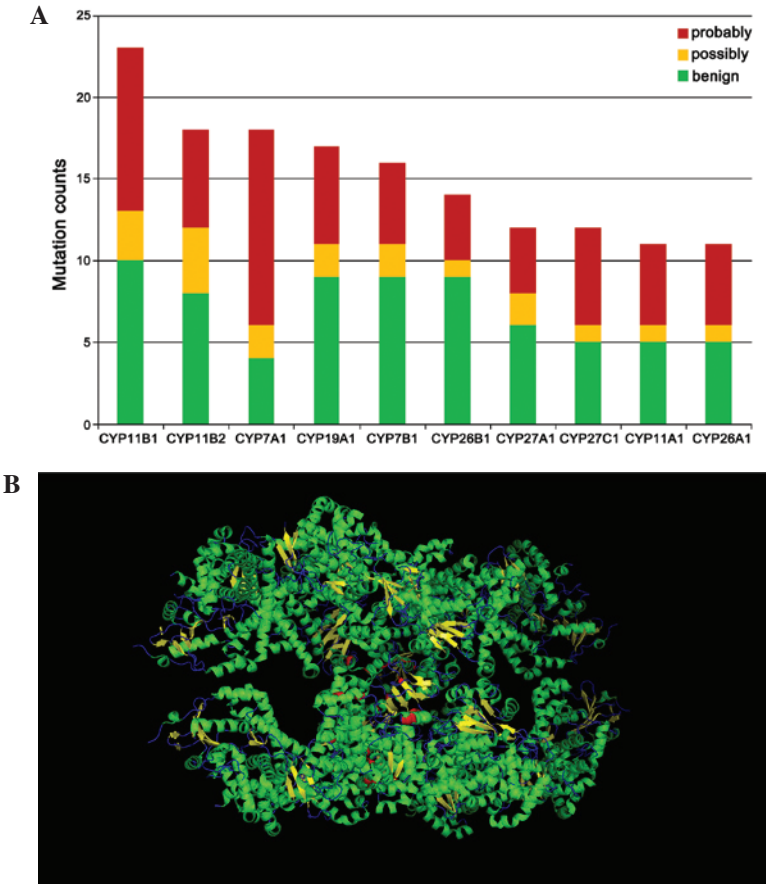

Figure 3. (A) Severity of CYP mutations were estimated in six cancer types using Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/). Top 10 gene in mutation number. (B) Overall protein secondary structure of CYP11B2, created using PyMOL (http://www.pymol.org/). Green represents α-helixes, yellow represents β-sheets, blue represents loops (neither α-helixes nor β-sheets) and red represents mutated sequences. CYPs, cytochromes P450.

and is controlled by anterior pituitary tropic hormones. Cortisol (a glucocorticoid steroid hormone) is composed by the zona fasciculata of the adrenal cortex. During stress or hypoglycemia, cortisol will be released to suppress the immune system, to increase blood glucose, to decrease bone formation and to support the metabolism of carbohydrates, fat and protein (16,17). Aldosterone (a mineralocorticoid steroid hormone) is formed by the zona glomerulosa of the adrenal cortex, and is important for blood pressure regulation (18). Blood pressure is managed by processes that occur in the distal convoluted tubules and collecting ducts of the nephron, which encourage the reabsorption of ions and water, the secretion of potassium, the conservation of sodium, and the increase in water retention, blood volume and blood pressure (18).

Discussion

The present study revealed that low CYP11A1 expression is common in several cancers, and CYP11B1 and CYP11B2 exhibit the highest number of mutations in these cancers. Approximately 80% of these mutations may alter the function of the CYP11B1 and CYP11B2 proteins. The findings of the present study indicate that the CYP11 family is commonly involved with a wide variety of cancers.

Decreased expression and mutations of the CYP11 family may influence the biosynthesis of steroid hormones. In recent years, the majority of studies of steroid hormones have been performed in breast cancer patients (19-22). However, studies have investigated the role of steroid hormones in prostate (23), lung (24), endometrial (25), colon (26) and liver cancer (27). Steroid hormones have been demonstrated to activate focal adhesion kinase, which regulates early actin reorganization in colon cancer cells (28). Steroid hormones were not previously considered to be involved with lung function (29); however, numerous studies have reported that steroid hormones are important in normal lung development and function (30) and in the pathogenesis of pulmonary diseases, including lung cancer (31-33). A study of prostate cancer validated the hypothesis that the biosynthesis of steroid hormones downstream of CYPs contributes to the progression of castration-resistant prostate cancer (34). Steroid hormones may also be utilized for the treatment of endometrial cancer. For example, progestin therapy has been demonstrated as a viable treatment option for type 1 endometrial cancer (35). These studies support the results of the present study, which indicated that steroid hormones are extremely important in numerous cancer types. In conclusion, the CYP11 family, which may affect steroid biosynthesis, is commonly involved in various types of cancer. The present study provides novel ideas that indicate that, with the development of technology, the CYP11 family could used as biomarker or drug target in the future research of cancers. Additional data and experiments will help to determine whether the genes of CYP11 family may be used as biomarkers in the diagnosis of various types of cancer. The method of computer-aided drug design may be used to simulate the interaction between the CYP11 family and chemical molecules, which will verify whether the CYP11 family could become drug targets.

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