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Expression of Cytochrome P450 in non-small cell lung cancer

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1. ABSTRACT

Lung cancer accounts for most of cancer-related deaths in both men and women. Lung cancer is also associated with cigarette smoking that exposes the individual to carcinogenic chemicals. Normally, CYP enzymes (cytochrome P450s) metabolize carcinogens to inactive derivatives, however, occasionally the action of CYP enzymes leads to development of more potent carcinogens. In addition to the metabolism of carcinogenic compounds, CYP enzymes are also involved in the activation and/or inactivation of agents, which are used in the treatment of lung cancer. Therefore, the local level of CYP enzymes in lung cancer and surrounding tissues could be an important determinant in the efficacy of anticancer drugs. Furthermore, the expression of CYP19 (aromatase), estrogen synthesis P450, was found in more than 80% of non-small cell lung cancers. Lung cancer was also found to frequently express CYP24A1 that converts 1 alpha, 25-dihydroxyvitamin D3 to its inactive 24-hydroxylated derivatives. The understanding of the local expression of CYP enzymes in tumor tissues is important in the development of better treatment for lung cancer and a standardized treatment, tailor-made, for individual patients.

2. INTRODUCTION

Lung cancer is the leading cause of cancer mortality in developed countries including Canada, France, Italy, Japan, UK, and USA (1). There are two broad types of lung tumor, usually classified as small cell lung cancer (SCLC), accounting for about 20% of cases, and non-small cell lung cancer (NSCLC), the most common form of lung cancer, accounting for up to 75% of all cases. There are three types of NSCLC: squamous cell carcinoma, adenocarcinoma, and large cell carcinoma (2). Current clinicopathological staging systems have the advantage of standardized criteria for assessing tumor stage, and a relationship between advancing tumor stage and poor prognosis has been established for NSCLC. However, these staging systems have not led to clear criteria for therapy selection in individual patients with NSCLC. The concept of therapy based on anatomical location, such as staging systems, is poorly associated with metabolic characteristics of individual tumor tissues (3).

The cytochrome P450 (CYP) family is a large group of constitutive and inducible haem-containing enzymes that catalyze the mono-oxygenation reaction using molecular oxygen and equivalent electrons from NADPH

via NADPH-dependent P450 reductase. CYPs play a central role in the oxidative metabolism of a diverse range of xenobiotics. Many P450 substrates are carcinogenic, while other substrates are anticancer drugs. CYPs therefore have various potentially important roles in tumor biology (4). Although the liver is the major organ that expresses most CYPs metabolizing exogenous chemicals, recent development in quantitative and qualitative detection methods of mRNA and proteins has enabled us to find that many organs and tissues as well as different types of tumors also express several CYPs (5). The increased CYP expression in tumors is frequently observed and is important not only for understanding of tumor development and progression but also for the efficient management of lung cancer with anticancer drugs. In the metabolism of anticancer drugs, CYPs are prominent players that enhance or diminish the anticancer function of therapeutic agents. The presence of individual forms of CYPs has been investigated in lung tumor for better understanding of the intra-tumor metabolism of anticancer agents (5, 6), suggesting the association of CYP expression in lung cancer with prognosis of patients.

Therefore, investigations of the tumor-specific CYP expression will provide the basis for the development of novel diagnostic and therapeutic strategies (4). In addition to the metabolism of carcinogens and anticancer agents, CYP19 catalyzing estrogen biosynthesis and CYP24 involved in vitamin $D3_3$ metabolism has been detected in lung cancer tissues, suggesting that CYP19 and CYP24 could be new therapeutic targets for the management of lung cancer.

3. EXPRESSION OF CYPs INVOLVED IN DRUG METABOLISM IN NON-SMALL CELL LUNG CANCER

Cytochrome P450 (CYP) enzymes expressed in human lungs can metabolize a variety of xenobiotics, drugs, and endogenous compounds (7). Metabolism of these substrates may lead to their detoxification or activation and may affect the homeostasis of the lung, its susceptibility to disease, response to therapy, and clinical prognosis (7). To better understand the importance of drug-metabolizing enzymes in carcinogenesis and anticancer drug sensitivity of human NSCLC, the study of the main drug-metabolizing enzyme systems in lung tumors is needed (8).

CYP expression has been studied in a variety of human NSCLC as well as normal tissues. These studies used a variety methods, such as detection of enzyme activity assay (EAA), identification of proteins by immunohistochemistry (IHC), Western blot analysis (WB), tissue microarray (TM) and detection of mRNA by Northern blotting (NB), reverse transcriptional polymerase chain reaction (RT-PCR), real-time quantitative PCR (RTQPCR), and RNase protection assay (RNP). The results of CYP expression in NSCLC reported by various groups were summarized in Table 1.

The expression of CYP1A1 and CYP1B1 in lungs is transcriptionally up-regulated by activation of the aryl hydrocarbon receptor (AhR) through binding of ligands such as cigarette smoke components (9). CYP1A1 is the most intensively studied CYP enzyme in the human lung because CYP1A1 is a major enzyme involved in polycyclic aromatic hydrocarbon (PAH) metabolism and may play an important role in the development of lung cancer through the activation of pro-carcinogens. CYP1A1 mRNA was found in 23% (10/43) of lung cancers by NB (10) and in 16% (2/10) by RTQPCR (11). CYP1A1 expression was found in 20% of lung cancers (n=10) by WB (11), and in 44% of adenocarcinoma (n=48) and in 37% of adenocarcinoma (n=107) by IHC (1, 9).

CYP1B1 metabolizes carcinogens associated with tobacco use. This enzyme also plays the major role in the metabolism of 17 beta-estradiol because of its capacity to catalyze the 4-hydroxylation reaction of estrogens. Using WB and RTQPCR, Spivack *et al*. (11, 12) reported that CYP1B1 was commonly expressed in approximately 100% of lung cancers. CYP1B1 expression was found in 47% of NSCLC (n=89) (13) and in 49% of adenocarcinoma (n=107) by IHC (9). Because of the common expression in human lung, CYP1B1 is hypothesized to be an important phase I enzyme with respect to carcinogen metabolism in the human lung (12).

CYP2E1 metabolizes some tobacco-specific nitrosamines (14). Activated carcinogens by CYP2E1 are associated with the formation of reactive oxygen species that cause tissue injury (15). CYP2E1 expression was found in 46% of 28 lung cancers by IHC (16) and in 40% of 48 adenocarcinoma by IHC (1). Based on the results from RT-PCR, Raunio *et al*. (17) demonstrated that normal lung tissues also express CYP2E1 mRNA. CYP2A6 expression was found in 46% of adenocarcinoma (n=48) by IHC (1). CYP2B7 and CYP4B1 mRNA were also present in normal lung and cancer tissues by RNase protection assay (7). In addition, normal lung tissues were also found to express CYP1A1, CYP3A5, CYP2B7, CYP4B1, and CYP2F1 mRNA determined by RT-PCR (17).

Recently, CYP has been shown to be involved in the metabolism of several essential anticancer agents such as alkaloids, vinca alkaloids, antimetabolites, and agents used for molecular targeting therapy and hormonal treatment. Table 2 shows relationship between anticancer drug and CYP metabolism. The information was obtained from manufacturer's documents attached with anticancer drugs. For instance, CYP3A enzymes not only inactivate the major anticancer drugs, alkaloids and vinca alkaloids as well as tamoxifen, but also activate some anticancer prodrugs, such as cyclophosphamide and ifosphamide (18, 19).

Since CYP3A consists of four subfamily members, CYP3A4, CYP3A5, CYP3A7, and CYP3A43, and since antibodies available from commercial sources usually cross-react with all CYP3A member proteins, the proteins detected with antibodies are described as CYP3A. CYP3A expression was demonstrated in both tumor and

Table 1. Expression of *CYP* genes in non-small cell lung cancer

Lung Cancer	Tumor tissue	
	Protein (rate) (reference)	RNA (rate) (reference)
CYP1A1	WB (Lower in tumor than normal tissue) (8) WB (20%) (11) IHC (44% of adenocarcinoma) (1) IHC (37% of adenocarcinoma) (9)	NB (23%) (10) RTQPCR (16%) (11)
CYP1B1	WB (100%) (12) WB (100%) (11) WB (100%) (13) IHC (47%) (13) IHC (49% of adenocarcinoma) (9)	RTQPCR (100%) (12) RTQPCR (80%) (11)
CYP2A6	IHC (46% of adenocarcinoma) (1)	
CYP2B7		RNP (100%) (7)
CYP2E1	IHC (46%) (16) IHC (40% of adenocarcinoma) (1)	
CYP3A	IHC (25%) (21) IHC (100%) (20) IHC (40% of adenocarcinoma) (1)	
CYP3A4	TM (3)	
CYP3A5	TM (3)	RT-PCR (50%) (20)
CYP3A7		RT-PCR (13%) (20)
CYP4B1		RNP (100%) (7)
CYP19	IHC (86%) (31)	
CYP24		RT-PCR (56%) (37)
CYP24A1		RT-PCR (38)

Items is % positive rate in tumor tissue or positive level. **WB**: Western blot analysis, **IHC**: Immunohistochemistry, **TM**: Tissue microarray , **EAA**: Enzyme activity assay, **RT-PCR**: Reverse transcriptase-polymerase, chain reaction, **RTQPCR:** Real-time quantitative polymerase chain reaction, **NB**: Northern blot analysis, **RNP**: RNase protection

Table 2. Relationship between anticancer drug and CYP metabolism.

Anticancer drug	CYP metabolism	
	Activation	Inactivation
Alkaloid		
Irinotecan		CYP3A4
Paclitaxel		CYP2C8, CYP3A4
Docetaxel		CYP3A4
Vinca alkaloids		
Vincristin		CYP3A4
Vinorelbine		CYP3A4
Antimetabolite		
Tegafur	CYP2A6	
Alkylating agent		
Cyclophosphamid	CYP2B6, CYP2C8, CYP2C9, CYP3A4, CYP2A6	
Molecular targeting therapy		
Gefitinib		CYP2D6, CYP3A4
Hormonal treatment		
Tamoxifen	CYP2B6, CYP2C9, CYP2D6, CYP3A4	CYP3A4

Data are extracted from the instruction for use of anticancer drugs provided by manufacturers

normal tissues by IHC (20). CYP3A expression was found in 40% of 48 adenocarcinoma by IHC (1). CYP3A4 has been shown to catalyze the activation of the prodrug ifosphamide, raising the possibility that ifosphamide could be activated in tumor tissues expressing this enzyme (18). CYP3A5 catalyzes the activation of the anticancer prodrugs cyclophosphamide and ifosphamide. CYP3A5 mRNA was found in all of eight lung cancers, and CYP3A4 mRNA in one of eight lung cancers (20). Eight of the 32 (25%) cases of lung cancer showed expression of CYP3A by IHC (21). Both CYP3A4 and CYP3A5 mRNA (22) as well as proteins (17, 23) were also identified in normal lung tissues. Local activation of carcinogens by CYP3A may take place in pulmonary carcinomas and surrounding normal tissues. PAH-DNA adduct levels was reported to have a positive correlation with the amount of CYP3A5 in alveolar macrophages of smokers (24, 25). Cyclophosphamide is also activated by CYP2B6 (Table 2). Utilizing adenoviral expression system, Tychopoulos *et al*. (26) investigated the effects of overexpression of CYP2B6 and P450-reductase fusion protein on the toxicity of cyclophosphamide in several pulmonary tumor cell lines. They showed a considerable enhancement of cyclophosphamide toxicity by the expression of the CYP2B6-reductase fusion protein, clearly indicating that CYP2B6 can activate cyclophosphamide. Similarly, other CYP enzymes could be involved in both activation of pro-anticancer drugs and inactivation of anticancer drugs. Cellular spectrum of CYP expression in pulmonary tumors should therefore be useful for application of CYP-dependent metabolisms for molecular targeting therapy. When the CYP enzymes catalyzing activation and/or inactivation of anticancer drugs are expressed in cancer tissues, the CYP-mediated drug metabolisms in tumors may be an important clinical factor in tumor sensitivity to these anticancer drugs.

4. EXPRESSION OF CYP19 (AROMATASE) IN LUNG CANCER

The number of female deaths from pulmonary adenocarcinoma is increasing (27), possibly associated with

estrogen function in the lung. However, the potential function of exogenous and endogenous estrogens in lung cancer development, especially adenocarcinoma in women, has been poorly investigated. Estrogens are involved in the differentiation and maturation of normal lungs (28), while they also stimulate the growth and progression of lung tumors (29) through the action of estrogen receptor (ER) (30). This steroidal growth-stimulatory pathway in tumors may be promoted by the expression and activity of aromatase (CYP19), an estrogen synthesis P450 (31). Aromatase (CYP19) synthesizes estrogens in adrenals and gonads as well as extragonadal tissues, including brain, skin, adipose, and lung tissues (31, 32). Using immunohistochemical staining, Weinberg *et al*. investigated the expression of CYP19 in lung cancer (n = 53). They reported that CYP19 had been detected in 86% of NSCLC, and that the CYP19 enzyme expressed in the tumors was biologically active (Table 1) (31). Utilizing a human lung cancer xenograph model system, Mah *et al*. reported the stimulatory effect of aromatase and estrogens on tumor growth (33). Therefore, therapeutic targeting of NSCLC to block estrogen signaling pathway may provide new options for the treatment of NSCLC patients (31). Hormonal treatment, such as tamoxifen, could be used for NSCLC patients. Tamoxifen binds to the estrogen receptor and blocks the estrogen function. Therefore, tamoxifen is used for the treatment of estrogen receptor-positive breast cancer (34) and also used as a preventative agent in women who are at an increased risk of developing breast cancer (35). Tamoxifen is extensively metabolized in the human liver. The formation of 4-hydroxytamoxifen, a potent anti-estrogen with high affinity for the estrogen receptor, is related to the therapeutic benefit achieved by the tamoxifen treatment. CYP2B6, CYP2C9, CYP2D6, and CYP3A4 enzymes are capable of catalyzing the 4-hydroxylation of tamoxifen as shown in Table 2. Although CYP3A4 enzyme is important for the activation of tamoxifen, CYP3A4 also converts tamoxifen to alpha-hydroxytamoxifen that is associated with an increased risk of endometrial cancer (36). Alternatively, third generation of aromatase inhibitors, anastrozole, letrozole, and exemestane, have proved very efficacious in the management of hormone-dependent breast cancer in post-menopausal women and also in prevention of recurrence. The aromatase inhibitors have also proved superior to tamoxifen for the treatment of breast cancer. Therefore, patients with aromatase-positive NSCLC tumor might be good candidates for the targeted treatment with aromatase inhibitors.

5. INVOLVEMENT OF VITAMIN D3 AND CYP24A1 IN LUNG CANCER

1 alpha, 25-dihydroxyvitamin D3 (1, 25-OH D3) and its analogues display potent antiproliferative activity in a variety of tumor mediated by vitamin D receptor (VDR) are currently under investigation in clinical trials in cancer (37, 38). Vitamin D3 synthesized by the action of light exposure in the skin is converted to 25-hydroxyvitamin D3 by CYP27A1 (vitamin D3 25-hydroxylase P450) in the liver and further converted to 1α, 25-dihydroxyvitamn D3, the active form of vitamin D3, by CYP27B1 (25-hydroxyvitamin D3 1 alpha-hydroxylase P450) in the kidney (39-42). In the target tissues of 1, 25-OH D3 such as kidney, small intestine, and bones, CYP24A1 (1, 25-hidroxyvitamin D3 24-hydroxylase) converts 1, 25-OH D3 to its inactive 24-hydroxyl derivatives (43, 44). The biosynthesis and inactivation pathways of 1, 25-OH D3 are important in the bone formation and maintenance, and thus these pathways have been intensively investigated as putative therapeutic targets for the treatment of osteoporosis. In addition to the involvement in bone formation and maintenance, the metabolism of vitamin D3 is also associated with tumor proliferation. The inactivation of 1, 25-OH D3 by CYP24A1 expressed in tumor tissues is associated with poor prognosis of some human cancers (38). Upon RT-PCR, CYP24A1 expression was detected in 10/18 lung cancers (37) and up-regulated CYP24A1 mRNA expression was also reported in lung cancers (Table 1) (38). The increased CYP24A1 expression observed in lung tumors should restrict 1, 25-OH D3 activity (37), suggesting that CYP24A1 could be an alternative target gene for the management of lung cancer.

6. PERSPECTIVES

Since various tumors express a variety of CYP enzymes, CYP may be good markers for the determination of quality of lung cancer (1). These drug-metabolizing CYP enzymes could reflect differences occurring after malignant transformation and may play a role in the sensitivity of tumor tissues to anticancer drugs. This encourages us to make efforts for the discovery of potential drugs that are specifically activated by CYP constitutively expressed in tumors. As summarized in Figure 1, there may be four directions for investigations that can be considered: A, since carcinogens are activated and/or inactivated by CYP enzymes, in depth study of local expression of CYPs and their involvement in the metabolisms of carcinogens will help for better understanding of the mechanisms of carcinogenesis. B, the spectrum of CYP expression in lung tumors may be useful tumor markers for classification and diagnosis of tumors relevant to the management of lung cancer. C, based on the data of CYP expression and metabolisms by the enzymes in lung tumors, new molecular targeting therapy could also be developed with novel agents that are specifically activated in individual lung cancers. D, CYP-dependent metabolisms of anticancer drugs are involved in both activation and inactivation of these agents. Therefore, the spectrum of CYP expression in lung tumors will provide information for use of therapeutic agents in a tailor-made fashion, in which we could choose an agent efficiently activated but not easily inactivated for individual patients. Furthermore, recent observations in the expression of aromatase and CYP24 in lung tumor tissues suggest possibilities that aromatase-specific inhibitors and CYP24-specific inhibitors may provide alternative therapeutic agents for better management of lung cancer. When 3-dimensional structures of

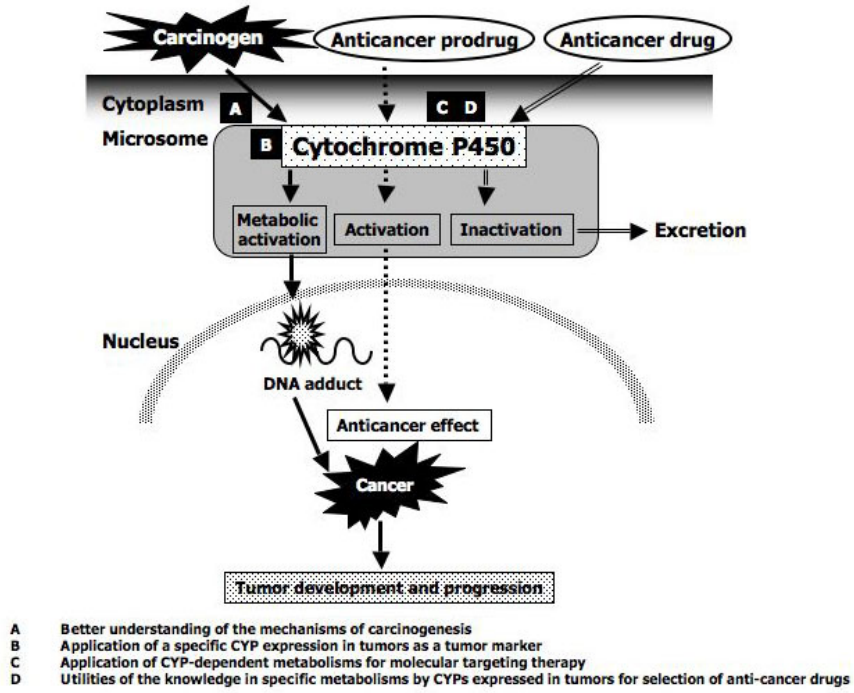

Figure 1. Cytochrome P450 (CYP) expressed in non-small cell lung cancer (NSCLC). CYP enzymes expressed in NSCLC play roles in the activation of carcinogens and pro-anticancer drugs as well as inactivation of carcinogenic agents and anticancer drugs. Information of CYP expression spectrum in individual tumors may be useful and essential in clinical applications for the better management of lung cancer.

aromatase and CYP24 become available in future, the structure-based design of inhibitors may enable us to create a therapeutic agent that specifically binds both aromatase and CYP24 and inhibits their enzymatic reactions. Thus, studies of the local expression of CYP in tumor tissues will provide insights into mechanisms of carcinogenesis and intratumoral metabolisms of anticancer drugs, which may enable us to develop the present order-made therapy to the tailor-made therapy.

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Abbreviations: CYP: cytochrome P450, SCLC: small cell lung cancer, NSCLC: non-small cell lung cancer, NADPH: nicotinamide adenine dinucleotide phosphate, EAA: enzyme activity assay, IHC: immunohistochemistry, WB: Western blot analysis, TM: tissue microarray, NB: Northern blotting, RT-PCR: reverse transcriptional polymerase chain reaction, RTQPCR: real-time quantitative PCR, RNP: RNase protection assay, AhR: aryl hydrocarbon receptor, PAH: polycyclic aromatic hydrocarbon, ER: estrogen receptor, VDR: vitamin D receptor

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