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Cytochrome P450 in non-small cell lung cancer related to exogenous chemical metabolism

Tsunehiro Oyama [1, 2], Hidetaka Uramoto [3], Norio Kagawa [4], Takashi Yoshimatsu [5], Toshihiro Osaki [6], Ryoichi Nakanishi [7], Hisao Nagaya [2], Kazuhiro Kaneko [8], Manabu Muto [9], Toshihiro Kawamoto [10], Fumihiro Tanaka [3], Akinobu Gotoh [2]

[1]Medical center for respiratory disease, Nishinihon Hospital, 3-20-1, Hattanda, Kumamoto, 861-8034, Japan, [2]Laboratory of Cell and Gene Therapy, Institute for Advanced Medical Sciences, Hyogo College of Medicine, 1-1, Mukogawa-cho, Nishinomiya, Hyogo, 663-8501 Japan, [3]Second Department of Surgery, University of Occupational and Environmental Health, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu, 807-8555, Japan, [4] Global COE, Nagoya University School of Medicine, 65, Tsurumai-cho, Showa-ku, Nagoya, Aichi, 466-8550, Japan,[5]Department of Thoracic Surgery, Fukuoka-Wajiro Hospital, 2-2-75, Wajirogaoka, Higashi-ku, Fukuoka, 811-0213, Japan, [6]Department of Chest Surgery, Iizuka Hospital, 3-83, Yoshio-machi, Iizuka, 820-8505, Japan, [7]Department of Thoracic Surgery, Shin-Kokura Hospital, Federation of National Public Service Personnel Mutual Aid Associations, 1-3-1, Kanada, Kokurakita-ku, Kitakyushu, 803-8505, Japan, [8]Department of Gastroenterology and GI Oncology, National Cancer Center Hospital East, 6-5-1, Kashiwanoha, Kashiwa, Chiba, 277-8577, Japan [9]Department of Gastroenterology and Hepatology, Graduate School of Medicine, 54, Shougoin Kawahara-cho, Sakyou-ku, Kyoto University, Kyoto, 606-8507, Japan, [10]Department of Environmental Health, University of Occupational and Environmental Health, 1-1, Iseigaoka, Yahatanishi-ku, Kitakyushu, 807-8555, Japan

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1. ABSTRACT

The occurrence of lung cancer is associated with smoking, which exposes smokers to a series of carcinogenic chemicals. CYP (cytochrome P450) usually metabolizes carcinogens to their inactive derivatives, but occasionally convert the chemicals to more potent carcinogens. In addition to the metabolism of carcinogenic compounds, CYP also participates in the activation and/or inactivation of anti-carcinogenic agents, suggesting that the local CYP expression in lung cancer and surrounding tissues could be an important determinant of efficacy of anticancer drugs. Furthermore, CYP19 (aromatase), estrogen synthase P450, expressed in more than 80% of non-small cell lung cancers. It suggests an association between estrogens and cancer development, which makes aromatase an attractive therapeutic target for the treatment of lung cancer. 1α,25-Dihydroxyvitamin D3 has an inhibitory effect on the proliferation of cancer tissues, and is converted to its inactive 24-hydroxylated derivatives by CYP24, which is frequently expressed in lung cancer tissues. Therefore, understanding the CYP expression in tumor tissues is important in developing better therapies for lung cancer, and may lead us to standardized, "tailor-made" therapies for individuals.

2. INTRODUCTION

Lung cancer is the leading cause of cancer mortality in developed countries, including Canada, France, Italy, the UK, the USA, and Japan (1). Lung tumors are classified into two broad classes, namely, small cell lung cancer (SCLC), accounting for about 15% of the cases, and non-small cell lung cancer (NSCLC), which is the most common form of lung cancer, accounting for up to 85% of all cases (2, 3). 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. Using the staging systems, the 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 the metabolic characteristics of individual tumor tissues (4).

The cytochrome P450 (CYP) family is a multi-gene superfamily comprising constitutive and inducible heme-containing mono-oxygenases (P450), in which the

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

Lung Cancer	Tumor tissue	
	Protein	**RNA**
CYP1A1	WB (Lower in tumor than normal tissue) (12) WB (20%) (15) IHC (44% of adenocarcinoma) (7) IHC (37% of adenocarcinoma) (13)	NB (23%) (14) RTQPCR (16%) (15)
CYP1B	WB (100%) (16) WB (100%) (15) WB (100%) (17) IHC (47%) (17) IHC (49% of adenocarcinoma) (13)	RTQPCR (100%) (16) RTQPCR (80%) (15)
CYP2A6	IHC (46% of adenocarcinoma) (7)	
CYP2A13	IHC (100% of adenocarcinoma) (18) IHC (93% of squamous cell carcinoma) (18)	
CYP2B7		RNP (100%) (11)
CYP2E1	IHC (46%) (27) IHC (40% of adenocarcinoma) (7)	
CYP3A	IHC (25%) (22) IHC (100%) (26) IHC (40% of adenocarcinoma) (7)	
CYP3A4	TM (4)	
CYP3A5	TM (4)	RT-PCR (50%) (26)
CYP3A7		RT-PCR (13%) (26)
CYP4B1		RNP (100%) (11)
CYP19	IHC (86%) (36)	
CYP24		RT-PCR (56%) (44)
CYP24A1		RT-PCR (43)

Superscript reveals the number of reference. Parentheses indicate % 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

characteristic absorption peak of the reduced CO-complex occurs at 450 nm. The CYP family is also a large group of enzymes that catalyze the mono-oxygenation reaction using molecular oxygen and equivalent electrons from NADPH *via* NADPH-dependent P450 reductase. The CYP system plays important roles in the metabolism and excretion of endogenous and exogenous compounds, including different carcinogens such as polycyclic aromatic hydrocarbons, heterocyclic amines, nitrosamines, azo dyes, and alkylating agents (5, 6). Metabolic intermediates of the compounds produced by CYP1A1, CYP2A6, CYP2E1, and CYP3A4/5 in this pathway are often highly active, and are associated with the initiation and promotion of tumor development and progression (5-7).

Many P450 substrates are carcinogens, while other substrates are anticancer drugs. CYPs, therefore, have potentially important roles in tumor biology (8). Although the liver is the major organ to express CYPs that metabolize exogenous chemicals, recent developments in quantitative and qualitative methods for mRNA and protein analyses have shown that many organs and tissues, as well as different types of tumors, express several CYPs (9). Increased CYP expression is frequently observed in tumors, and is important not only for understanding tumor development and progression, but for efficient management of lung cancer also. In the metabolism of anticancer drugs, CYPs are prominent players that enhance or diminish the anticancer function of therapeutic agents. The presence of individual CYPs in lung tumors has been investigated to better understand the intra-tumor metabolism of anticancer agents (9, 10); the results strongly suggest that CYP expression in lung cancer is associated with the prognosis of patients.

Therefore, investigations of tumor-specific CYP expression will provide a basis for the development of novel diagnostic and therapeutic strategies (8). In addition to the metabolism of carcinogens and anticancer agents, CYP19 catalysis of estrogen biosynthesis and CYP24 involvement in vitamin D3 metabolism have been detected in lung cancer tissues, suggesting that CYP19 and CYP24 could be new therapeutic targets for the management of lung cancer.

3. CYP EXPRESSION IN NON-SMALL CELL LUNG CANCER

Cytochrome P450 (CYP) enzymes expressed in human lungs can metabolize a variety of xenobiotics, drugs, and endogenous compounds (11). Metabolism of these substrates leads to their detoxification and/or activation, which may affect the homeostasis of the lung as well as its susceptibility to disease, response to therapy, and clinical prognosis (11). To better understand the importance of drug-metabolizing enzymes in carcinogenesis and the anticancer drug sensitivity of human NSCLC, it is necessary to study the major drug-metabolizing enzyme systems in lung tumors (12).

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

The expression of CYP1A1 and CYP1B1 in lungs is transcriptionally up-regulated by the activation of the aryl hydrocarbon receptor (AhR) through binding of ligands such as cigarette smoke components (13). CYP1A1 is the most intensively studied CYP enzyme in the human lung because CYP1A1 is the major enzyme metabolizing polycyclic aromatic hydrocarbon (PAH) 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) cases of lung cancer by NB (14) and in 16% (2/10) cases of lung cancer by RTQPCR (15).

Table 2. Relationship between anticancer drug and CYP metabolism

Anticancer drug		CYP metabolism	
Generic name	**Trade name**	**Activation**	**Inactivation**
Alkaloids			
Irinotecan	Campto, Topotecin		CYP3A4
Paclitaxel	Taxol		CYP2C8, CYP3A4
Docetaxel	Taxotere		CYP3A4
Vinca alkaloids			
Vincristin	Oncovin		CYP3A4
Vinorelbine	Navelbine		CYP3A4
Antimetabolites			
Gemcitabine	Gemzar	-	-
Pemetrexed	Alimta	-	-
Tegafur-Uracil	UFT	CYP2A6, CYP1A2, CYP2E1	
Tegafur-Gimeracil-Oteracil	TS1	CYP2A6, CYP1A2, CYP2E1	
Alkylating agents			
Cyclophosphamid	Endoxan	CYP2B6, CYP2C8, CYP2C9,CYP3A4, CYP2A6	
Molecular targeting agents			
Gefitinib	Iressa		CYP2D6, CYP3A4
Erlotinib	Tarceva		CYP3A4, CYP1A2
Crizotinib	-		CYP3A4 (susp)*
Bevacizumab	Avastin	-	-

These data are extracted from the attachments of anticancer drugs provided by manufacturers

CYP1A1 expression was found in 20% cases of lung cancer (n = 10) by WB (15) and in 44% cases of adenocarcinoma (n = 48) and 37% cases of adenocarcinoma (n = 107) by IHC (7, 13).

CYP1B1 metabolizes the carcinogens associated with tobacco use. This enzyme plays a major role in converting estradiol to its 4-hydroxyl derivative, which is a putative carcinogenic metabolite of estrogen. Using WB and RTQPCR, Spivack *et al.* (15, 16) reported that CYP1B1 was commonly expressed in approximately 100% cases of lung cancer. CYP1B1 expression was found in 47% cases of NSCLC (n = 89) (17) and in 49% cases of adenocarcinoma (n = 107) by IHC (13). Because of its common expression in human lung, CYP1B1 is hypothesized to be an important phase I enzyme with respect to carcinogen metabolism in the human lung (16).

CYP2A13, which is preferentially expressed in the respiratory tract, is the most efficient enzyme for the metabolic activation of tobacco-specific nitrosamines, such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The relevance of CYP2A13 in carcinogenicity and toxicity in the respiratory tract has been suggested (18). CYP2A13 expression was found in 100% cases of adenocarcinoma (n = 15) and in 93% cases of squamous cell carcinoma (n = 15) by IHC (18). A variant enzyme produced by the genetic polymorphism *CYP2A13*2* was found to be associated with substantially reduced risk for smoking-related lung adenocarcinoma (19), also suggesting the importance of CYP2A13 in the development of lung adenocarcinoma.

CYP2E1 metabolizes some tobacco-specific nitrosamines (20). Carcinogens activated by CYP2E1 are associated with the formation of reactive oxygen species that cause tissue injury (21). CYP2E1 expression was found in 46% of 28 cases of lung cancer by IHC (22) and in 40% of 48 cases of adenocarcinoma by IHC (7). Based on the results from RT-PCR, Raunio *et al.* (23) demonstrated that normal lung tissues also express CYP2E1 mRNA. CYP2A6 expression was found in 46% cases of adenocarcinoma (n = 48) by IHC (7). CYP2B7 and CYP4B1 mRNA were also present in the normal lung and cancer tissues analyzed by RNase protection assay (11). In addition, normal lung tissues were also found to express CYP1A1, CYP3A5, CYP2B7, CYP4B1, and CYP2F1 mRNA, as determined by RT-PCR (23).

Recently, CYPs have been shown to metabolize several essential anticancer agents such as alkaloids, vinca alkaloids, antimetabolites, alkylating agents, as well as the agents used for molecular targeting therapy. Table 2 shows the relationship between anticancer drugs and CYP metabolism. The information was obtained from the manufacturers' documents attached with the anticancer drugs. For instance, CYP3A enzymes not only inactivate the major anticancer drugs, namely, alkaloids and vinca alkaloids, as well as molecular targeting agents, but also activate some anticancer prodrugs such as cyclophosphamide and ifosfamide (24, 25).

Since CYP3A consists of four subfamily members, namely, CYP3A4, CYP3A5, CYP3A7, and CYP3A43, and since the 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 normal tissues by IHC (26). CYP3A expression was found in 40% of 48 cases of adenocarcinoma by IHC (7). CYP3A4 has been shown to catalyze the activation of the prodrug ifosfamide, raising the possibility that ifosfamide could be activated in tumor tissues expressing this enzyme (25). CYP3A5 catalyzes the activation of cyclophosphamide and ifosfamide. CYP3A5 mRNA was found in all of eight lung cancers, and CYP3A4 mRNA, in one of eight lung cancers (26). Eight of the 32 (25%) cases of lung cancer showed the expression of CYP3A by IHC (27). Both CYP3A4 and CYP3A5 mRNA (28) and proteins (23, 29) were also expressed in normal lung tissues. Local activation of carcinogens by CYP3A may occur in pulmonary carcinomas and surrounding normal tissues (30). Cyclophosphamide is also activated by CYP2B6 (Table 2). Utilizing an adenoviral expression system, Tychopoulos *et al.* (31) investigated the effects of overexpression of a CYP2B6 and P450-reductase fusion protein on the toxicity of cyclophosphamide in several pulmonary tumor cell lines. Cyclophosphamide toxicity was considerably enhancedby the expression of the

CYP2B6-reductase fusion protein, clearly indicating that CYP2B6 can activate cyclophosphamide.

4. EXPRESSION OF CYP19 (AROMATASE) IN LUNG CANCER

The number of deaths in the female population with pulmonary adenocarcinoma is increasing (32), and it is possibly associated with estrogen function in the lung (30). Estrogen is involved in the differentiation and maturation of normal lungs (33), while it also stimulates the growth and progression of lung tumors (34) through the action of the estrogen receptor (ER) (35). This steroidal growth-stimulatory pathway in tumors may be promoted by the expression and activity of aromatase (CYP19), an estrogen synthase P450 (36). Aromatase (CYP19) synthesizes the estrogen in the adrenals and gonads as well as in the extragonadal tissues, including the brain, skin, and adipose, pancreatic, and lung tissues (36-39). The gene expression of aromatase is regulated by a number of tissue-specific promoters located in the 93-kb 5′-flanking region of the aromatase gene. Demura *et al.* (40) reported that normal lung tissues dominantly used the promoter I.4, which is also used in the skin, and adipose and vascular tissues. In tumor tissues, alternative promoters I.1 (placenta-specific), I.3 (adipose tissue- and ovary-specific), and I.7 (aorta-specific) were utilized at a higher level compared to the normal tissues, although the promoter I.4 was still dominantly used (40). Using immunohistochemical staining, Weinberg *et al.* investigated the expression of CYP19 in lung cancer (n = 53) and reported that CYP19 was detected in 86% of NSCLC, and that the CYP19 enzyme expressed in the tumors was biologically active (Table 1) (36). Utilizing a human lung cancer xenograft model system, Mah *et al.* reported a stimulatory effect of aromatase and estrogen on tumor growth (41). Márquez-Garbán *et al.* (42) also treated lung NSCLC xenografts *in vivo* with an aromatase inhibitor alone and a combination of cisplatin/aromatase inhibitors, and showed a considerable reduction in tumor progression compared to the paired control. Therefore, therapeutic targeting of NSCLC to block the estrogen-signaling pathway may provide new options for the treatment of NSCLC patients (36).

5. INVOLVEMENT OF VITAMIN D3 AND CYP24A1 IN LUNG CANCER

1α,25-Dihydroxyvitamin D3 (1,25-OH D3) and its analogs display potent anti-proliferative activity, mediated by the vitamin D receptor (VDR), in a variety of tumors, and are currently under investigation in clinical trials for anticancer agents (43, 44). Vitamin D3, synthesized by the exposure of skin to light, is converted to 25-hydroxyvitamin D3 by CYP27A1 (vitamin D3 25-hydroxylase P450) in the liver, and further to 1α,25-dihydroxyvitamn D3, the active form of vitamin D3, by CYP27B1 (25-hydroxyvitamin D3 1α-hydroxylase P450) in the kidney (45-48). In 1,25-OH D3-target tissues such as the kidney, small intestine, and bones, CYP24A1 (1,25-hydroxyvitamin D3 24-hydroxylase) converts 1,25-OH D3 to its inactive 24-hydroxyl derivatives (49, 50). The biosynthesis and inactivation pathways of 1,25-OH D3 are important in bone formation and maintenance, and thus, these pathways have been intensively investigated as putative therapeutic targets for the treatment of osteoporosis. In addition to its 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 (43). Upon RT-PCR, CYP24A1 expression was detected in 10 of 18 cases of lung cancer (44), and up-regulated CYP24A1 mRNA expression was also reported in lung cancers (Table 1) (43). The increased CYP24A1 expression observed in lung tumors should restrict 1,25-OH D3 activity (44), 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, CYPs may be good markers for the determination of the quality of lung cancer (7). Drug-metabolizing CYP enzymes could reflect the differences occurring after malignant transformation, and may play a role in the sensitivity of tumor tissues to anticancer drugs. Therefore, we endeavor to discover potential drugs that are specifically activated by the CYPs constitutively expressed in tumors. As summarized in Figure 1, three CYP metabolic pathways can be defined according to the substrate, such as carcinogens, anticancer prodrugs, and anticancer drugs. As shown in Figure 1A, tobacco smoke contains many carcinogens and pro-carcinogens such as benzo(a)pyrene and nitrosamine. Carcinogens such as benzo(a)pyrene are metabolized by phase I enzymes, including CYP family enzymes, and converted to inactive metabolites by phase II enzymes. Benzo(a)pyrene itself, for instance, does not exhibit carcinogenicity, but undergoes metabolic activation by the phase I enzyme CYP1A1 into the diol epoxide, which is mostly converted to an inactive metabolite by the phase II enzyme glutathione S-transferase (GST), particularly the Mu class of GST (GSTM1) (51). However, this epoxide is extremely reactive, and can pave its way into the nucleus to form DNA adducts, which exhibit strong carcinogenicity. CYP-mediated benzo(a)pyrene DNA adducts can cause specific mutations in the p53 gene (52). In Figure 1B, some anticancer drugs are inactivated by CYP. In Figure 1C, some anticancer prodrugs are activated by CYP.

Since substrates such as carcinogens, anticancer prodrugs, and anticancer drugs are activated and/or inactivated by CYP enzymes, in depth study of the local expression of CYP enzymes and their involvement in the metabolism of carcinogens will help explain the mechanisms of carcinogenesis. Based on CYP expression and metabolism by CYP enzymes in lung tumors, new molecular targeting therapy could also be developed with novel agents that are specifically activated in individual lung cancers. As described in this review, aromatase- and CYP24A1-specific inhibitors could also provide novel treatments for non-small cell lung cancer. Therefore, the spectrum of CYP expression in lung tumors will provide

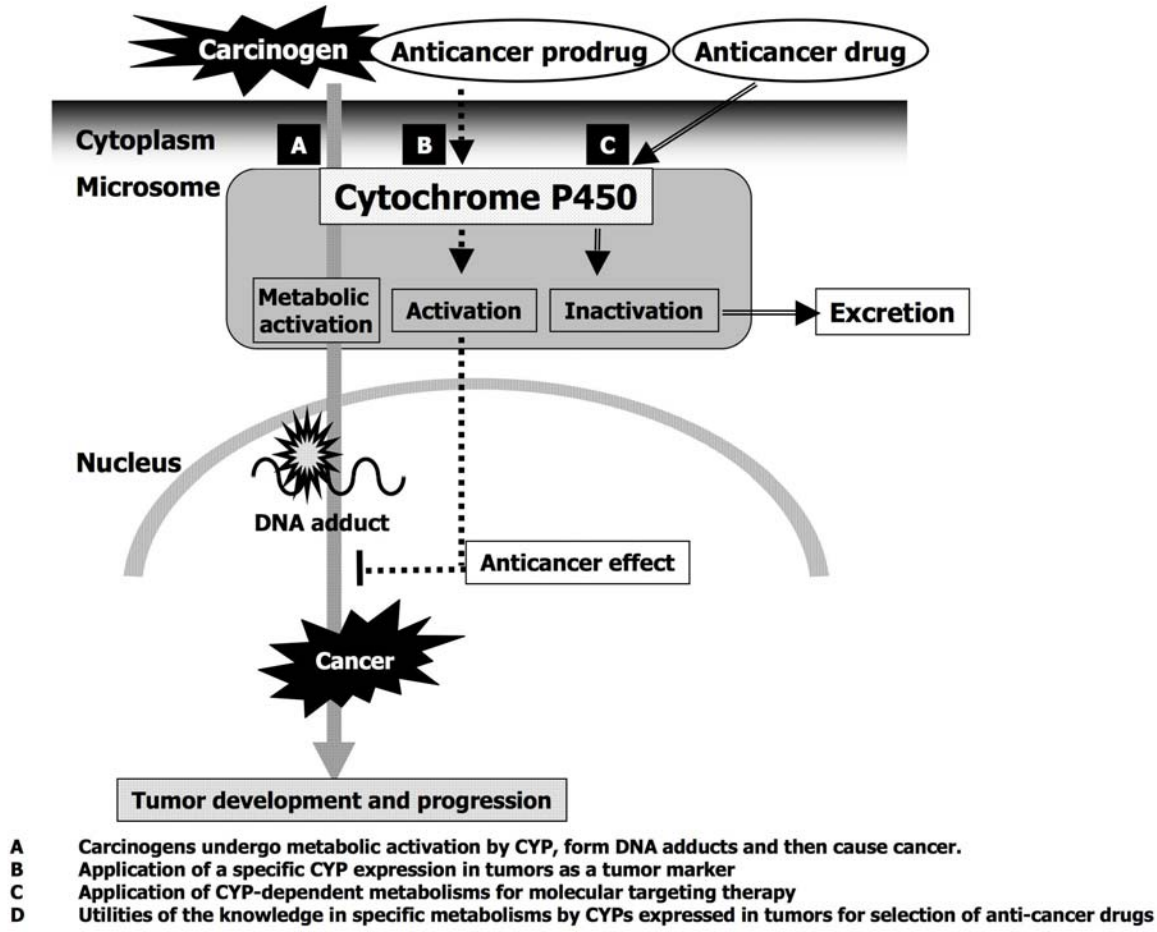

Figure 1. Cytochrome P450 (CYP) expressed in non-small cell lung cancer (NSCLC). CYP enzymes expressed in NSCLC play roles in the activation of carcinogens and pro-anticancer drugs as well as inactivation of carcinogenic agents and anticancer drugs. Information of CYP expression spectrum in individual tumors may be useful and essential in clinical applications for the better management of lung cancer. *: Crizotinib suspected to be inactivated by CYP3A4.

information for the use of therapeutic agents in a "tailor-made" fashion, whereby we can choose an agent that is efficiently activated, but not easily inactivated, for each individual patient. The spectrum of CYP expression in lung tumors may provide useful tumor markers for the classification and diagnosis of tumors relevant to the management of lung cancer. Thus, studying the local expression of CYP in tumor tissues will provide insights into the mechanisms of carcinogenesis and the intratumoral metabolism of anticancer drugs, which may enable us to developthe present "order-made" therapy to "tailor-made" therapy.

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8. REFERENCES

1. A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu and M. J. Thun: Cancer statistics, 2009. *CA Cancer J Clin*, 59(4), 225-49 (2009)

2. E. Brambilla, W. D. Travis, T. V. Colby, B. Corrin and Y. Shimosato: The new World Health Organization classification of lung tumours. *Eur Respir J*, 18(6), 1059-68 (2001)

3. D. S. Ettinger: Overview and state of the art in the management of lung cancer. *Oncology (Williston Park)*, 18(7 Suppl 4), 3-9 (2004)

4. W. Zhang, W. D. Shannon, J. Duncan, G. L. Scheffer, R. J. Scheper and H. L. McLeod: Expression of drug pathway proteins is independent of tumour type. *J Pathol*, 209(2), 213-9 (2006)

5. F. J. Gonzalez and H. V. Gelboin: Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. *Drug Metab Rev*, 26(1-2), 165-83 (1994)

6. K. F. Windmill, R. A. McKinnon, X. Zhu, A. Gaedigk, D. M. Grant and M. E. McManus: The role of xenobiotic metabolizing enzymes in arylamine toxicity and carcinogenesis: functional and localization studies. *Mutat Res*, 376(1-2), 153-60 (1997)

7. T. Oyama, K. Sugio, H. Uramoto, T. Kawamoto, N. Kagawa, S. Nadaf, D. Carbone and K. Yasumoto: Cytochrome P450 expression (CYP) in non-small cell lung cancer. *Front Biosci*, 12, 2299-308 (2007)

8. G. I. Murray: The role of cytochrome P450 in tumour development and progression and its potential in therapy. *J Pathol*, 192(4), 419-26 (2000)

9. T. Oyama, N. Kagawa, N. Kunugita, K. Kitagawa, M. Ogawa, T. Yamaguchi, R. Suzuki, T. Kinaga, Y. Yashima, S. Ozaki, T. Isse, Y. D. Kim, H. Kim and T. Kawamoto: Expression of cytochrome P450 in tumor tissues and its association with cancer development. *Front Biosci*, 9, 1967-76 (2004)

10. N. Gharavi and A. O. El-Kadi: Expression of cytochrome P450 in lung tumor. *Curr Drug Metab*, 5(2), 203-10 (2004)

11. M. Czerwinski, T. L. McLemore, H. V. Gelboin and F. J. Gonzalez: Quantification of CYP2B7, CYP4B1, and CYPOR messenger RNAs in normal human lung and lung tumors. *Cancer Res*, 54(4), 1085-91 (1994)

12. C. Toussaint, N. Albin, L. Massaad, D. Grunenwald, O. Parise, Jr., J. Morizet, A. Gouyette and G. G. Chabot: Main drug- and carcinogen-metabolizing enzyme systems in human non-small cell lung cancer and peritumoral tissues. *Cancer Res*, 53(19), 4608-12 (1993)

13. J. T. Chang, H. Chang, P. H. Chen, S. L. Lin and P. Lin: Requirement of aryl hydrocarbon receptor overexpression for CYP1B1 up-regulation and cell growth in human lung adenocarcinomas. *Clin Cancer Res*, 13(1), 38-45 (2007)

14. T. L. McLemore, S. Adelberg, M. C. Liu, N. A. McMahon, S. J. Yu, W. C. Hubbard, M. Czerwinski, T. G. Wood, R. Storeng, R. A. Lubet and *et al.*: Expression of CYP1A1 gene in patients with lung cancer: evidence for cigarette smoke-induced gene expression in normal lung tissue and for altered gene regulation in primary pulmonary carcinomas. *J Natl Cancer Inst*, 82(16), 1333-9 (1990)

15. S. D. Spivack, G. J. Hurteau, M. J. Fasco and L. S. Kaminsky: Phase I and II carcinogen metabolism gene expression in human lung tissue and tumors. *Clin Cancer Res*, 9(16 Pt 1), 6002-11 (2003)

16. S. D. Spivack, G. J. Hurteau, A. A. Reilly, K. M. Aldous, X. Ding and L. S. Kaminsky: CYP1B1 expression in human lung. *Drug Metab Dispos*, 29(6), 916-22 (2001)

17. P. Lin, H. Chang, W. L. Ho, M. H. Wu and J. M. Su: Association of aryl hydrocarbon receptor and cytochrome P4501B1 expressions in human non-small cell lung cancers. *Lung Cancer*, 42(3), 255-61 (2003)

18. T. Fukami, M. Nakajima, I. Matsumoto, Y. Zen, M. Oda and T. Yokoi: Immunohistochemical analysis of CYP2A13 in various types of human lung cancers. *Cancer Sci*, 101(4), 1024-8 (2010)

19. H. Wang, W. Tan, B. Hao, X. Miao, G. Zhou, F. He and D. Lin: Substantial reduction in risk of lung adenocarcinoma associated with genetic polymorphism in CYP2A13, the most active cytochrome P450 for the metabolic activation of tobacco-specific carcinogen NNK. *Cancer Res*, 63(22), 8057-61 (2003)

20. H. Kushida, K. Fujita, A. Suzuki, M. Yamada, T. Endo, T. Nohmi and T. Kamataki: Metabolic activation of N-alkylnitrosamines in genetically engineered Salmonella typhimurium expressing CYP2E1 or CYP2A6 together with human NADPH-cytochrome P450 reductase. *Carcinogenesis*, 21(6), 1227-32 (2000)

21. E. Albano, P. Clot, M. Morimoto, A. Tomasi, M. Ingelman-Sundberg and S. W. French: Role of cytochrome P4502E1-dependent formation of hydroxyethyl free radical in the development of liver damage in rats intragastrically fed with ethanol. *Hepatology*, 23(1), 155-63 (1996)

22. K. T. Kivisto, A. Linder, G. Friedel, P. Beaune, C. Belloc, H. K. Kroemer and P. Fritz: Immunohistochemical localization of cytochrome P450 2E1 in human pulmonary carcinoma and normal bronchial tissue. *Virchows Arch*, 426(3), 243-7 (1995)

23. H. Raunio, J. Hakkola, J. Hukkanen, O. Pelkonen, R. Edwards, A. Boobis and S. Anttila: Expression of xenobiotic-metabolizing cytochrome P450s in human pulmonary tissues. *Arch Toxicol Suppl*, 20, 465-9 (1998)

24. F. P. Guengerich: Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu Rev Pharmacol Toxicol*, 39, 1-17 (1999)

25. K. T. Kivisto, H. K. Kroemer and M. Eichelbaum: The role of human cytochrome P450 enzymes in the metabolism of anticancer agents: implications for drug interactions. *Br J Clin Pharmacol*, 40(6), 523-30 (1995)

26. K. T. Kivisto, E. U. Griese, P. Fritz, A. Linder, J. Hakkola, H. Raunio, P. Beaune and H. K. Kroemer: Expression of cytochrome P 450 3A enzymes in human lung: a combined RT-PCR and immunohistochemical analysis of normal tissue and lung tumours. *Naunyn Schmiedebergs Arch Pharmacol*, 353(2), 207-12 (1996)

27. K. T. Kivisto, P. Fritz, A. Linder, G. Friedel, P. Beaune and H. K. Kroemer: Immunohistochemical localization of cytochrome P450 3A in human pulmonary carcinomas and normal bronchial tissue. *Histochem Cell Biol*, 103(1), 25-9 (1995)

28. S. Anttila, J. Hukkanen, J. Hakkola, T. Stjernvall, P. Beaune, R. J. Edwards, A. R. Boobis, O. Pelkonen and H. Raunio: Expression and localization of CYP3A4 and CYP3A5 in human lung. *Am J Respir Cell Mol Biol*, 16(3), 242-9 (1997)

29. J. Hukkanen, O. Pelkonen, J. Hakkola and H. Raunio: Expression and regulation of xenobiotic-metabolizing cytochrome P450 (CYP) enzymes in human lung. *Crit Rev Toxicol*, 32(5), 391-411 (2002)

30. T. Oyama, K. Sugio, T. Isse, A. Matsumoto, N. Nose, H. Uramoto, T. Nozoe, M. Morita, N. Kagawa, T. Osaki, M. Muto, K. Yasumoto and T. Kawamoto: Expression of cytochrome P450 in non-small cell lung cancer. *Front Biosci*, 13, 5787-93 (2008)

31. M. Tychopoulos, L. Corcos, P. Genne, P. Beaune and I. de Waziers: A virus-directed enzyme prodrug therapy (VDEPT) strategy for lung cancer using a CYP2B6/NADPH-cytochrome P450 reductase fusion protein. *Cancer Gene Ther*, 12(5), 497-508 (2005)

32. A. M. Coscio and J. Garst: Lung cancer in women. *Curr Oncol Rep*, 8(4), 248-51 (2006)

33. C. Patrone, T. N. Cassel, K. Pettersson, Y. S. Piao, G. Cheng, P. Ciana, A. Maggi, M. Warner, J. A. Gustafsson and M. Nord: Regulation of postnatal lung development and homeostasis by estrogen receptor beta. *Mol Cell Biol*, 23(23), 8542-52 (2003)

34. R. J. Pietras, D. C. Marquez, H. W. Chen, E. Tsai, O. Weinberg and M. Fishbein: Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. *Steroids*, 70(5-7), 372-81 (2005)

35. T. Oyama, M. Morita, T. Isse, N. Kagawa, S. Nakata, T. So, M. Mizukami, Y. Ichiki, K. Ono, M. Sugaya, H. Uramoto, T. Yoshimatsu, T. Hanagiri, K. Sugio, T. Kawamoto and K. Yasumoto: Immunohistochemical evaluation of cytochrome P450 (CYP) and p53 in breast cancer. *Front Biosci*, 10, 1156-61 (2005)

36. O. K. Weinberg, D. C. Marquez-Garban, M. C. Fishbein, L. Goodglick, H. J. Garban, S. M. Dubinett and R. J. Pietras: Aromatase inhibitors in human lung cancer therapy. *Cancer Res*, 65(24), 11287-91 (2005)

37. N. Kagawa, H. Hori, M. R. Waterman and S. Yoshioka: Characterization of stable human aromatase expressed in E. coli. *Steroids*, 69(4), 235-43 (2004)

38. N. Kagawa: Efficient expression of human aromatase (CYP19) in E. coli. *Methods Mol Biol*, 705, 109-22 (2011)

39. W. C. Boon, J. D. Chow and E. R. Simpson: The multiple roles of estrogens and the enzyme aromatase. *Prog Brain Res*, 181, 209-32 (2010)

40. M. Demura, Y. Demura, S. Ameshima, T. Ishizaki, M. Sasaki, I. Miyamori, M. Yamagishi, Y. Takeda and S. E. Bulun: Changes in aromatase (CYP19) gene promoter usage in non-small cell lung cancer. *Lung Cancer*, 73(3), 289-93 (2011)

41. V. Mah, D. B. Seligson, A. Li, D. C. Marquez, Wistuba, II, Y. Elshimali, M. C. Fishbein, D. Chia, R. J. Pietras and L. Goodglick: Aromatase expression predicts survival in women with early-stage non small cell lung cancer. *Cancer Res*, 67(21), 10484-90 (2007)

42. D. C. Marquez-Garban, H. W. Chen, L. Goodglick, M. C. Fishbein and R. J. Pietras: Targeting aromatase and estrogen signaling in human non-small cell lung cancer. *Ann N Y Acad Sci*, 1155, 194-205 (2009)

43. M. G. Anderson, M. Nakane, X. Ruan, P. E. Kroeger and J. R. Wu-Wong: Expression of VDR and CYP24A1 mRNA in human tumors. *Cancer Chemother Pharmacol*, 57(2), 234-40 (2006)

44. R. A. Parise, M. J. Egorin, B. Kanterewicz, M. Taimi, M. Petkovich, A. M. Lew, S. S. Chuang, M. Nichols, T. El-Hefnawy and P. A. Hershberger: CYP24, the enzyme that catabolizes the antiproliferative agent vitamin D, is increased in lung cancer. *Int J Cancer*, 119(8), 1819-28 (2006)

45. N. Sawada, T. Sakaki, S. Kitanaka, S. Kato and K. Inouye: Structure-function analysis of CYP27B1 and CYP27A1. Studies on mutants from patients with vitamin D-dependent rickets type I (VDDR-I) and cerebrotendinous xanthomatosis (CTX). *Eur J Biochem*, 268(24), 6607-15 (2001)

46. E. Uchida, N. Kagawa, T. Sakaki, N. Urushino, N. Sawada, M. Kamakura, M. Ohta, S. Kato and K. Inouye: Purification and characterization of mouse CYP27B1 overproduced by an Escherichia coli system coexpressing molecular chaperonins GroEL/ES. *Biochem Biophys Res Commun*, 323(2), 505-11 (2004)

47. N. Urushino, K. Yamamoto, N. Kagawa, S. Ikushiro, M. Kamakura, S. Yamada, S. Kato, K. Inouye and T. Sakaki: Interaction between mitochondrial CYP27B1 and adrenodoxin: role of arginine 458 of mouse CYP27B1. *Biochemistry*, 45(14), 4405-12 (2006)

48. K. Yamamoto, E. Uchida, N. Urushino, T. Sakaki, N. Kagawa, N. Sawada, M. Kamakura, S. Kato, K. Inouye and S. Yamada: Identification of the amino acid residue of CYP27B1 responsible for binding of 25-hydroxyvitamin D3 whose mutation causes vitamin D-dependent rickets type 1. *J Biol Chem*, 280(34), 30511-6 (2005)

49. T. Sakaki, N. Kagawa, K. Yamamoto and K. Inouye: Metabolism of vitamin D3 by cytochromes P450. *Front Biosci*, 10, 119-34 (2005)

50. T. Sakaki, N. Sawada, K. Komai, S. Shiozawa, S. Yamada, K. Yamamoto, Y. Ohyama and K. Inouye: Dual metabolic pathway of 25-hydroxyvitamin D3 catalyzed by human CYP24. *Eur J Biochem*, 267(20), 6158-65 (2000)

51. T. Oyama, T. Mitsudomi, T. Kawamoto, A. Ogami, T. Osaki, Y. Kodama and K. Yasumoto: Detection of CYP1A1 gene polymorphism using designed RFLP and distributions of CYP1A1 genotypes in Japanese. *Int Arch Occup Environ Health*, 67(4), 253-6 (1995)

52. T. Oyama, T. Kawamoto, T. Mizoue, K. Nishida, T. Osaki, K. Sugio, K. Yasumoto and T. Mitsudomi: p53 mutations of lung cancer are not significantly affected by CYP1A1 or GSTM1 polymorphisms. *Int J Oncol*, 11, 305-309 (1997)

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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Send correspondence to: Tsunehiro Oyama, Medical center for respiratory disease, Nishinihon Hospital, Kumamoto, 861-8034, Japan, Tel: 96-380-1111, Fax : 96-380-0539, E-mail: oyama@nishinihon.or.jp