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（計畫名稱）

二氫二醇去氫酶在肺癌癌細胞中的生理病理調控以及其與環氧仔、抗藥性、雄性激素受體和 HER2/neu 基因之交互作用

Pathophysiological regulation of dihydrodiol dehydrogenase expression in lung cancer cells, and the interactions with cyclooxygenases, drug resistance, androgen receptor and HER2/neu gene expressions

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中文摘要

本研究計畫是探討二氫二醇去氫仔在非小細胞肺癌細胞中的生理病理調控以及其與環氧仔、抗藥性、雄性激素受體和 HER2/neu 基因之交互作用；我們使用免疫組織染色法作為偵測基因在癌細胞中表現的主要方法，基因表現則更進一步以免疫轉漬和反轉錄聚合仔鏈反應的方法確定。二氫二醇去氫仔與其他基因之表現以及病人之病理生理因子之相關性與存活率，則以統計方法分析；我們發現二氫二醇去氫仔在非小細胞肺癌病人中的表現約為 83.2% (317/381)；在轉移之淋巴腺上則為 80.7% (176/218)。二氫二醇去氫仔之表現與其他基因的相互關係則是與穀光羥　、nm23 和抗藥性成反比；與環氧仔並無對等關聯。在 304 名男性患者中僅有 85 名患者之癌細胞中表現雄性受體，其表現也是和二氫二醇去氫仔之表現無對等關聯。

關鍵字：二氫二醇去氫仔、肺癌細胞、基因表現、環氧仔、抗藥性、雄性激素受體和 HER2/neu

Abstract

In this study we investigated the correlation among expressions of dihydrodiol dehydrogenase (DDH) and other inflammation- and drug resistance-associated genes in patients with non-small cell lung cancer (NSCLC). By using immunohistochemistry, we measured expressions of DDH and other genes in 381 patients with NSCLC. Gene expressions were confirmed by immunoblotting and reverse transcription-polymerase chain reaction. Relation between DDH expression and clinicopathological parameters was analyzed by statistical analysis. Difference of survivals between different groups was compared by a log rank test. DDH overexpression was detected in 83.2% of pathological sections (317/381) and in 80.7% of metastatic lymph nodes (176/218). DDH expression was inversely correlated with GST, nm-23 and MDR-1expressions, but not related to COX-2 expression. Interestingly, among 304 male patients, only 85 (27.9%) samples expressed androgen receptor (AR) in the tumor cells. Compared with patients with DDH overexpression in tumors, patients with low DDH expression had significantly lower incidence of lymph node metastasis and tumor recurrences. Interestingly, survival was also significantly better in patients with low DDH expression than in those with DDH overexpression ($P = 0.0017$). In conclusions, for patients with NSCLC, DDH overexpression was correlated with smoking habit, tumor stages, lymphovascular invasion and poor prognosis.

Abbreviations used in this paper: COX-2, cyclooxygenase-2; DDH, dihydrodiol dehydrogenase; NSCLC, non-small cell lung cancer; GST, glutathione-S-transferase; AR, androgen receptor.

目　　錄

INTRODUCTION

Lung cancer is one of the leading causes of cancer death worldwide. In Taiwan, the annual mortality rate of lung cancer is about 20 percent of total cancer-related deaths.[1] In the United States, the annual death rate of lung cancer is approximately 30 percent, and the toll exceeds the death numbers of breast, prostate and colon cancer combined.[2] Most of the patients who die are at the late stage of the disease when they are diagnosed. However, some patients who are diagnosed at the early stage, and undergo the curative resection still die of cancer due to the early recurrence and metastasis.[3] Even the use of the most advanced chemotherapy, containing cyclophosphamide, cisplatin, doxorubicin, gemcitabine, taxanes, vindesin and/or vinorelbine, could only prolong patients' median survival to 8 months (ranged from 6.2 to 8.5 months), and increase patients' one-year survival rate to 33 percent.[4,5] It is quite obvious that resistance to anticancer drugs could play an important role with this respect. On the other hand, epidemiological evidences, by which the use of nonsteroidal antiinflammatory drugs (NSAIDs), e.g., aspirin, indomethacin, and sulindac, could reduce not only the risk of NSCLC, but also the risks of gastric, colorectal, esophageal and breast cancers,[6,7] further support the concept that inflammation and carcinogenesis are intimately related. The detailed mechanism of how NSAIDs reduce the risk of cancer development, however, remains to be determined. It is clear though that cyclooxygenases, the key enzymes in converting arachidonic acid to prostanoids, are targets of NSAIDs.[8]

In fact, beside cyclooxygenases, interconversion of prostanoids could be catalyzed by prostaglandin (PG) F synthase, a member of aldo-keto reductase family.[9,10] Recently, by using differential display to examine specimens of non-small cell lung cancer (NSCLC) and lung cancer cell lines, we have identified overexpression of dihydrodiol dehydrogenase (DDH) that was not detected in the corresponding normal lung tissue.[11] DDH is also a member of aldo-keto reductase superfamily, which mediates NADP-associated oxidation of *trans*-dihydrodiols to the corresponding catechols.[12] In human liver, at least four multiple forms of the enzyme (DDH1-DDH4) have been identified in the cytoplasm with monomeric mass of 36 kDa.[13] Among these hepatic DDHs, DDH1 and DDH2 could also exhibit PGF synthase activity by converting PGD_2 into $9\alpha,11\beta$-PGF_2.[14] Detection of DDH overexpression in NSCLC cells would then provide an alternative link between chronic inflammation and carcinogenesis of lung, and, possibly, the disease manifestation of NSCLC.

It should be noted that in the liver, DDH normally converts mutagenic polycyclic aromatic hydrocarbon (PAH) into catechol.[12,15] Oxidation of catechol could further form PAH *o*-quinones that can be rapidly conjugated with glutathione.[16,17] The lack of glutathione or the detoxification system for PAH was then implicated as one of the reasons for PAH-related carcinogenesis.[18-21] One of the few detoxification systems which have been identified in the lung is glutathione-S-transferase (GST). The enzyme catalyzes the reaction of glutathione with electrophiles. This system is important especially in the initiation of carcinogenesis, as relative forms of many carcinogens, including nitrosamines that are carcinogenic for the esophagus, are electrophilic.[22] Furthermore, results from the previous studies, which emphasized the genetic polymorphisms of (GST) in the high-risk areas of NSCLC, also showed that GST-null genotypes and GST-deficient phenotypes were more susceptible to the cancer development.[23-25]

In this study, we used an immunohistochemical method to determine the expressions of DDH and GST in surgical specimens from patients with NSCLC. Overexpression of DDH in NSCLC was further confirmed by in situ hybridization, immunoblotting and reverse transcription-polymerase chain reaction (RT-PCR). The correlation between clinicopathological parameters and DDH expression and the prognostic significance of DDH expression in patients with NSCLC were evaluated.

MATERIALS AND METHODS

Patients and Tissue Samples From 1986 to 1999, tissue specimens from 398 consecutive patients with newly diagnosed lung cancer were collected. All patients were with pathologically confirmed NSCLC. Stage of disease progression was classified according to the Unio International Contre Cancer system. Radical en bloc esophagectomy was done for every patient. The surgical mortality

of 17 patients was caused by pulmonary complications.[2] Thus, only 381 patients entered for prognosis analysis. For patients at stage II or beyond, postoperative adjuvant therapy was commenced within one month if patient's condition was suitable. Irradiation was prescribed for 60Gy (10 Gy/5 fractions/week). Those with distant metastasis were treated with chemotherapy.[26] After treatment, all patients were followed as routine. Written informed consent was obtained from every patient. Tumor recurrence and metastasis were identified when blood examination, biochemical studies, chest radiography, sonography of neck and abdomen, whole body bone radioisotopic can and computerized tomography scans of chest showed any suspected evidence of the disease.[2]

Immunohistochemical Staining and Immunoblotting Immunohistochemical staining was performed by an immunoperoxidase method as previously described.[11, 27] Antibodies for GST (π) and COX-2 were from Oxford Biomedical Res. (Oxford, MI), that for nm-23 was from Santacruz (CA, USA), and those for mdr-1 and HER2/neu were from Dako (Kyoto, Japan) respectively. The same antibodies were used for immunoblotting. Procedure for immunoblotting has been described previously.[11]

In Situ Hybridization As described previously, a nonisotopic method, with fluorescein isothiocyanate (FITC)-labeled DDH antisense oligonucleotides, was used to determine the expression of DDH mRNA.[11]

RNA Extraction, and Gene Amplification RNA extraction and gene amplification have been described previously.[11] Briefly, following RNA extraction, cDNA was synthesized by oligo dT primer and AMV reverse transcriptase. An aliquot of cDNA was then subjected to 35 cycles of polymerase chain reaction (PCR) using standard procedure denaturing at 94°C for 1 min, hybridizing at 52°C for 30 seconds, and elongating at 72°C for 1.2 min. The primer sequences for DDH were 5'-GTGTGAAGCTGAATGATGGTCA-3' (GenBank/ABO21654, nts 20-41) and 5'-TCTGATGCGCTGCTCATTGTAGCTC-3' (GenBank/ABO21654, nts 834-810). The primer sequences for GST were 5'-CAGAGGAGGTCGCAGTTCAG-3' (GenBank/M99422, nts 211-230), and 5'-CATCCCTTAGCCCAGTCAAG-3' (GenBank/M99422, nts 900-881). The amplified products were resolved in a 2.5% agarose-ethidium bromide gel. Specificity of the 815 base-pair DDH fragment was confirmed by DNA sequencing (Perkin-Elmer, Foster City, CA), and the nucleotide sequences were matched with the database listed in GenBank (http://www.ncbi.nlm.nih.gov/blast).[11]

Slide Evaluation. In each case, normal esophagus tissue served as internal negative control. Slides were read by three independent observers without clinicopathological knowledge. A specimen was considered positive if more than 10% of cancer cells were positively stained; and negative if less than 10% were positively stained.[11, 27]

Statistical Analysis. Relations between DDH overexpression and clinicopathological parameters were analyzed by Chi-Square test or *t* test respectively. When the expected number of any analysis cell was smaller than or equal to five cases, Fisher's exact test was used. Survival curves were plotted with method of Kaplan-Meier.[28] Statistical difference of survivals between different groups was compared by the log rank test. Statistical analysis was performed using SPSS statistical software (Chicago, IL).[29] Statistical significance was set at P value < 0.05.

RESULTS

As determined by immunohistochemistry, 317 patients (83.2%) were positive for DDH overexpression (Figure 1). DDH was also detected in 80.7% of metastatic lymph nodes (176/218), and DDH mRNA in surgical specimens was verified by ISH. However, expression of GST was only weakly detected in 60 specimens (15.7%), of which COX-2 and nm-23 were identified in 82 (21.5%) and 142 (37.2%) surgical samples respectively, and that of mdr-1 was in 218 pathological sections (57.2%) (Table 1). The correlation between COX-2 expression and DDH expression was not significant ($P = 0.072$). GST expression and DDH expression, on the other hand, were inversely correlated, to a statistically significant level ($P < 0.01$). Interestingly, expressions of DDH and nm23 were also inversely correlated ($P < 0.01$).

Among 317 patients with DDH overexpression, 90 (28.4%) had tumor recurrences, which

developed within 24 months after the operation. On the other hand, among 64 patients with low DDH expression, only 12 (18.8%) had tumor recurrences. The difference was significant ($P < 0.01$). By immunoblot analysis, overexpression of DDH was detected in eight of nine surgical specimens, and the results were verified in seven samples by RT-PCR. Nucleotide sequence of the DNA fragments from seventeen NSCLC specimens matched with that of DDH2: GenBank | AB031084 Homo sapiens aldo-keto reductase family 1, member C2 (dihydrodiol dehydrogenase 2; bile acid-binding protein) (AKR1C2), identities = 652/653 (99%). Nucleotide sequence of twenty-one samples matched with that of DDH1: GenBank | NM_001353.2| Homo sapiens aldo-keto reductase family 1, member C1 (dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase) (AKR1C1), identities = 749/768 (97%). Expression of GST detected by immunoblotting was decreased in these NSCLC specimens when compared with that of DDH. The results were consistent with those of immunohistochemical staining (Table 1). *In vitro*, expressions of these genes were further confirmed by RT-PCR (Figure 2). Interestingly, COX-2 gene expressions varied between squamous cell cancer cell lines and those of adenocarcinoma origin (Figure 3).

Clinically, the median follow-up period for the 381 patients was 43 months, ranging from 2.0 to 86 months. The mean age of the patients was 63.0 years, ranging from 42 to 75 years. In this study, 304 men and 77 women were enrolled, and 275 patients (72.2%) were smokers. Following surgery, 102 patients had evidence of tumor recurrence. Nonetheless, no significant difference was found between patients with and those without DDH overexpression according to the following clinicopathological parameters such as age, mitotic index and cell differentiation patterns (Table 1). On the other hand, statistical differences were found with respect to patients' gender, smoking habit, tumor stages and lymphovascular invasion. Male patients had a significantly higher incidence of DDH overexpression than female patients ($P < 0.05$). The survival of patients who overexpressed DDH was significantly poorer than that of patients with low DDH expression. The differences in cumulative survivals were significant ($P = 0.0017$).[11] Using univariate analysis, male gender, smoking habit, numbers of lymph node involvement, tumor stages and DDH overexpression were five important factors correlated with poor prognosis for patients with NSCLC.

DISCUSSION

The results presented above demonstrate that overexpression of DDH in NSCLC correlated with smoking habit, tumor stages, lymphovascular invasion and patients' survival. Patients with DDH overexpression in NSCLC cells have significantly higher incidence of the early tumor recurrences that are frequently associated with the poor prognosis. Expression of GST, in contrast, was decreased in NSCLC cells in these patients.

As noted previously, epidemiological data have indicated an increasing risk of developing lung cancer in a person who has GST-null genotype or GST-deficient phenotype[23-25] due to the lack of glutathione or GST for PAH detoxification.[18-21] On the other hand, the risk of lung cancer could be reduced by using clinically important NSAIDs to inhibit cyclooxygenase activity.[5, 30] Interestingly, Zimmermann *et al* have shown the correlation of COX-2 overexpression with the increased risk of cancer development.[30] They further proposed that COX-2 inhibitor could suppress PGE_2 synthesis, and inhibit cell proliferation by abrogating mitotic activity and by increasing apoptosis frequency in the responding cells. However, COX-2 expression was relatively heterogeneous among tumor populations, and the enzyme was not invariably found in every tumor nest pathologically. Our findings of the expression of DDH that has PGF synthase activity in NSCLC cells suggested a possible alternative source of prostaglandins that were essential for regulating cell proliferation. Interestingly, our results further demonstrated that DDH expression in NSCLC was positively related to COX-2 expression, but inversely correlated with GST expression. These evidences indicate that DDH overexpression may play an important role in lung carcinogenesis, as well as disease progression.

The impact of DDH and its product PGF on NSCLC cell proliferation remains to be clarified if this is the basis of disease manifestation. It should be noted that other explanations are possible. Correlation between DDH and COX-2 expressions indicated that these two enzymes might be under a common physiological regulation. Moreover, the results of statistical analysis, in which

expression of nm23 was correlated with that of DDH, supported our previous observations that tumor progression is a concerted process that includes a battery of genes expressing differentially at different stages of the disease. In particular, for patients with the advanced diseases in whom the pathophysiological variables would be far more than one single gene could dominate, these variables may involve IL-6,[31] sIL-2Rα,[32] DDH,[11] COX-2 and nm23.[33] The correlation of these variables is evaluated in an ongoing study. It is worth noting that overexpression of nm23-H1 in NSCLC was demonstrated to associate with the cisplatin-induced DNA damage.[34] Interestingly, our recent work on cisplatin-resistant ovarian cell lines also detected DDH overexpression.[35] Although the detailed network of gene expressions is yet to be clarified, the effect of DDH, nm23 and GST on drug resistance of lung cancer cells demands further investigation.

It is worth noting that nm23 protein is associated with multiple biological functions in cell proliferation and differentiation,[36, 37] such as enhancement of nucleoside diphosphate kinase (NDPK) activity, increase in the serine phosphorglation, TGFβ-induced growth inhibition of cells, and is a component in the ras-dependent signal transudation. However, the significance of nm23-H1 expression in human cancers may differ with the organs in which the tumor develops, or the histologic patterns of tumors. In fact, similar to our results, several previous investigators have demonstrated that increased expression of nm23 in cancer cells is associated with aggressive tumor growth or disease progression, such as breast,[38] neuroblastoma[39] gastric carcinoma[40, 41], thyroid carcinomas[42], head and neck carcinomas[43, 44] and lung cancer.[40, 45-47] Interestingly, we found a contrast result that the overall survival rate is positively correlated with the nm23-H1 expression, while all of our patients had received cisplatin-based chemotherapy for advanced or recurrent lesions. By examining the in-vitro sensitivity of various tumor cell lines to chemotherapeutic compounds, Fergnson *et al* indicated that sensitivity to cisplatin could be increased by nm23-transfected tumor cell lines.[48] Clinically, Scambia et al reported that the expression of nm23-H1 protein correlated inversely with the prognosis of patients with ovarian carcinoma after cisplatin-based chemotherapy.[49] It was then suggested that reduced expression of intracellular nm23-H1 in ESCC cells was associated with cisplatin resistance via the prevention of both nuclear and mitochondrial damage and recommended that it might be related to Na+, K-ATPase activity, which was responsible for intracellular cisplatin accumulation. It also was reported that nm23-H1is preferentially expressed in the S-phase of the cell cycle[36] and that cisplatin has the strongest cytotoxic effect on the cancer cells of the S-G2 phase.[50] Thus, another possibility is that nm23-H1 may play a role in cisplatin-induced cytotoxity via modulating the cell cycle. It should be seriously considered that when the nm23-H1 positive cancer cells are associated with a high malignant potential but more sensitive to cisplatin-based chemotherapy; then the residual cancer cells in the tumor after cisplatin-based chemotherapy could be those of less malignant potential. Thus, these patients can be alive with tumor and then improve their survival. However, the other roles of nm23-H1 in cisplatin-induced cytotoxicity remains to be further clarified.

CONCLUSIONS

At the present time, our results showed that DDH expression was frequently detected in the pathologic specimens of NSCLC patients and was correlated with smoking habit, tumor stages and lymphovascular invasion. Although there is not yet a clear explanation for the clinical correlation between increased DDH expression and disease progression, these observations would serve as a foundation to elucidate the mechanism by which expression of DDH in cancer cells is regulated pathologically.

REFERENCES

1. Annual reports of the Department of Health, the Executive Yuan, Republic of China, 2000
2. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH; The Eastern Cooperative Oncology Group. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 2002; 346: 92-98.
3. Pairolero PC, Williams DE, Bergstralh EJ, *et al*. Postsurgical stage I bronchogenic

carcinoma: morbid implications of recurrent disease. Ann Thorac Surg 1984;38: 331-336.
4. Rapp E, Pater JL, Willan A, *et al.* Chemotherapy can prolong survival in patients with advanced non-small-cell lung cancer - report of a Canadian multicenter randomized trial. J Clin Oncol 1988; 6: 633-641.
5. Grilli R, Oxman AD, Julian JA. Chemotherapy for advanced non-small-cell lung cancer: how much benefit is enough? J Clin Oncol 1993; 11: 1866-1872.
6. Thun MJ, Namboodiri MM, Calle EE, Flanders WD, Heath CW, Jr. Aspirin use and risk of fatal cancer. Cancer Res 1993;53:1322-1327.
7. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 1994;5:138-146.
8. Subbaramaiah K, Zakim D, Weksler BB, Dannenberg AJ. Inhibition of cyclooxygenase: a novel approach to cancer prevention. Proc Soc Exp Biol Med 1997;216:201-210.
9. Watanabe K, Fujii Y, Nakayama K, Ohkubo H, Kuramitsu S, Kagamiyama H, Nakanishi S, Hayaishi O. Structural similarity of bovine lung prostaglandin F synthase to lens epsilon-crystallin of the European common frog. Proc Natl Acad Sci U S A 1988;85:11-15.
10. Suzuki T, Fujii Y, Miyano M, Chen LY, Takahashi T, Watanabe K. cDNA cloning, expression, and mutagenesis study of liver-type prostaglandin F synthase. J Biol Chem 1999;274:241-248.
11. Hsu NY, Ho HC, Chow KC, Lin TY, Shih CS, Wang LS, Tsai CM. Overexpression of Dihydrodiol Dehydrogenase as a Prognostic Marker of Non-Small Cell Lung Cancer. Cancer Res 2001;61:2727-2731.
12. Vogel K, Bentley P, Platt KL, Oesch F. Rat liver cytoplasmic dihydrodiol dehydrogenase. J Biol Chem 1980;255:9621-9625.
13. Hara A, Taniguchi H, Nakayama T, Sawada H. Purification and properties of multiple forms of dihydrodiol dehydrogenase from human liver. J Biochem (Tokyo) 1990; 108:250-254.
14. Hara A, Matsuura K, Tamada Y, Sato K, Miyabe Y, Deyashiki Y, Ishida N. Relationship of human liver dihydrodiol dehydrogenases to hepatic bile-acid-binding protein and an oxidoreductase of human colon cells. Biochem J 1996;313:373-376.
15. Shou M, Harvey RG, Penning TM. Contribution of dihydrodiol dehydrogenase to the metabolism of (±)-*trans*-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in fortified rat liver subcellular fractions. Carcinogenesis 1992;13:1575-1582.
16. Glatt HR, Vogel K, Bentley P, Oesch F. Reduction of benzo(a)-pyrene mutagenicity by dihydrodiol dehydrogenase. Nature 1979;277:319-320.
17. Flowers-Geary L, Harvey RG, Penning TM. Cytotoxicity of polycyclic aromatic hydrocarbon *o*-quinones in rat and human hepatoma cells. Chem Res Toxicol 1993;6: 252-260.
18. Shen H, Kauvar L, Tew KD. Importance of glutathione and associated enzymes in drug response. Oncol Res 1997;9:295-302.
19. Penning TM, Sharp RB. Characterization of dihydrodiol dehydrogenase in human liver and lung. Carcinogenesis 1990;11:1203-1208.
20. Burczynski ME, Harvey RG, Penning TM. Expression and characterization of four recombinant human dihydrodiol dehydrogenase isoforms: oxidation of trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene to the activated o-quinone metabolite benzo[a]pyrene-7,8-dione. Biochemistry 1998;37:6781-6790.
21. Burczynski ME, Lin HK, Penning TM. Isoform-specific induction of a human aldo-keto reductase by polycyclic aromatic hydrocarbons (PAHs), electrophiles, and oxidative stress:implications for the alternative pathway of PAH activation catalyzed by human dihydrodiol dehydrogenase. Cancer Res 1999;59:607-614.
22. Sparnins VL, Chuan J, Wattenberg LW. Enhancement of glutathione S-transferase activity of the esophagus by phenols, lactones, and benzyl isothiocyanate. Cancer Res 1982;42:1205-1207.
23. Tan W, Song N, Wang GQ, Liu Q, Tang HJ, Kadlubar FF, Lin DX. Impact of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferases M1, T1, and P1 on susceptibility to esophageal cancer among high-risk individuals in China. Cancer Epidemiol Biomarkers Prev 2000;9:551-556.
24. Roth MJ, Dawsey SM, Wang G, Tangrea JA, Zhou B, Ratnasinghe D, Woodson KG, Olivero OA, Poirier MC, Frye BL, Taylor PR, Weston A. Association between GSTM1*0 and

squamous dysplasia of the esophagus in the high risk region of Linxian, China. Cancer Lett 2000;156:73-81.
25. van Lieshout EM, Roelofs HM, Dekker S, Mulder CJ, Wobbes T, Jansen JB, Peters WH. Polymorphic expression of the glutathione S-transferase P1 gene and its susceptibility to Barrett's esophagus and esophageal carcinoma. Cancer Res 1999;59:586-589.
26. Wang LS, Chi KH, Hu MH, Fahn HJ, Huang MH. Management for patients with advanced T4 epidermoid carcinoma of the esophagus. J Surg Oncol 1996;62:22-29.
27. Hsieh CC, Chow KC, Fahn HJ, Tsai CM, Li WY, Huang MH, Wang LS. Prognostic significance of HER-2/neu overexpression in stage I adenocarcinoma of lung. Ann Thorac Surg 1998;66:1159-1163.
28. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53: 457-481.
29. Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 1966;50:163-170.
30. Zimmermann KC, Sarbia M, Weber AA, Borchard F, Gabbert HE, SchrÖr K. Cyclooxygenase-2 expression in human esophageal carcinoma. Cancer Res 1999;59:198-204.
31. Wang LS, Chow KC, Wu CW. Expression and up-regulation of interleukin-6 in oesophageal carcinoma cells by n-sodium butyrate. Br J Cancer 1999;80:1617-1622.
32. Wang LS, Chow KC, Li WY, Liu CC, Wu YC, Huang MH. Clinical significance of serum soluble interleukin-2 receptor-alpha in esophageal squamous cell carcinoma. Clin Cancer Res 2000;6:1445-1451.
33. Hsu NY, Chow KC, Chen WJ, Lin CC, Chou FF, Chen CL. Expression of nm23 in the primary tumor and the metastatic regional lymph nodes of patients with gastric cardiac cancer. Clin Cancer Res 1999; 5:1752-1757.
34. Iizuka N, Hirose K, Noma T, Hazama S, Tangoku A, Hayashi H, Abe T, Yamamoto K, Oka M. The nm23-H1 gene as a predictor of sensitivity to chemotherapeutic agents in oesophageal squamous cell carcinoma. Br J Cancer 1999;81:469-475.
35. Deng HB, Parekh HK, Chow KC, Simpkins H. Increased expression of dihydrodiol dehydrogenase induces resistance to cisplatin in human ovarian carcinoma cells. J Biol Chem 2002;277:15035-15043.
36. Caligo MA, Cipollini G, Fiore L, Calvo S, Basolo F, Collecchi P, et al. Nm23-gene expression correlates with cell growth rate and s-phase. Int J Cancer. 1995;60:837-842.
37. Caligo MA, Cipollini G, Petrini M, Valentini P, Bevilacqua G. Down-regulation of nm23.H1 and c-myc genes during differentiation induced by 1,25 dihydroxyvitamin D3. Leukemia Res. 1996;20:161-167.
38. Sartre-Garau X, Lacombe ML, Jouve M, Veron M, Magdelenat H. Nucleoside diphosphate kinase/nm23 expression in breast cancer: lack of correlation with lymph node metastasis. Int J Cance.r 1994;50:157-160.
39. Hailat N, Kleim DR, Melhelm RF et al.: High levels of D19/nm23 protein in neuroblastoma are associated with advanced stage disease and with n-myc gene amplification. J Clin Invest. 1991;88:341-345.
40. Müller W, Schneiders A, Hommel G, Gabbert HE. Expression of nm23 in gastric carcinoma, association with tumor progression and poor prognosis. Cancer. 1998;83:2481-2487.
41. Nakamura T, Tabuchi Y, Ohno M. Relations of nm23 expression to clinico-pathologic variables and proliferative activity of gastric cancer lesions. Cancer Detection & Prevention. 1998;22:246-250.
42. Zou M, Shi Y, Al-Sedairy S, Farid NR. High levels of nm23 gene expression in advanced stage of thyroid carcinomas. Br J Cancer. 1993;68:385-388.
43. Song AU, Mais DD, Groo S, Wright JR, Yoshida GY. Expression of nm23 antimetastic gene product in head and neck squamous cell carcinoma. Otolaryngol Head Neck Surg. 2000;122:96-99.
44. Pavelić K, Kapitanović S, Radošević S, Bura M, Seiwerth S, Paveelić LJ, et al. Increased activity of nm23-h1 gene in squamous cell carcinoma of the head and neck is associated with advanced disease and poor prognosis. J Mol Med. 2000;78:111-118.
45. Huwer H, Engel M, Welter C, Dooley S, Kalweit G, Feindt P, et al. Squamous cell carcinoma

of the lung: does the nm23 gene expression correlate to the tumor stage? Thorac Cardiovasc Surgeon. 1994;42:298-301.
46. Bosnar MH, Pavelic K, Krizanac S, Slobodnjak Z, Pavelic J. Squamous cell lung carcinomas: the role of nm23-H1 gene. J Mol Med 1997;75:609-613.
47. Engel M, Theisinger B, Seib T, Seitz G, Huwer H, Zang KD, et al. High levels of nm23-H1 and nm23-H2 messenger RNA in human squamous-cell lung. Int J Cancer 1993;55:375-379.
48. Ferguson AW, Flatow U, MacDonaldd NJ, Larminat F, Bohr VA, Steeg PS. Increased sensitivity to cisplatin by nm23-transfected tumor cell lines. Cancer Res. 1996;56:2931-2935.
49. Scambiz G, Ferrandinaa G, Marone M, Benedetti PP, Giannitelli C, Piantelli M, et al. Nm23 in ovarian cancer: correlation with clinical outcome and other clinicopathologic and biochemical prognostic parameters. J Clin Oncol 1996;14:334-342.
50. Nguyen HN, Sevin BU, Averette HE, Perras J, Ramos R, Donato D, et al. Cell cycle perturbations of platinum derivatives on two ovarian cancer cell lines. Cancer Invest 1993;11:264-275.

LEGENDS

Figure 1 Expression of DDH in lung adenocarcinoma cells detected by immunohistochemistry.

Figure 2 *In vitro*, expressions of cyclooxygenase and the associated genes in lung adenocarcinoma cells were further confirmed by RT-PCR.

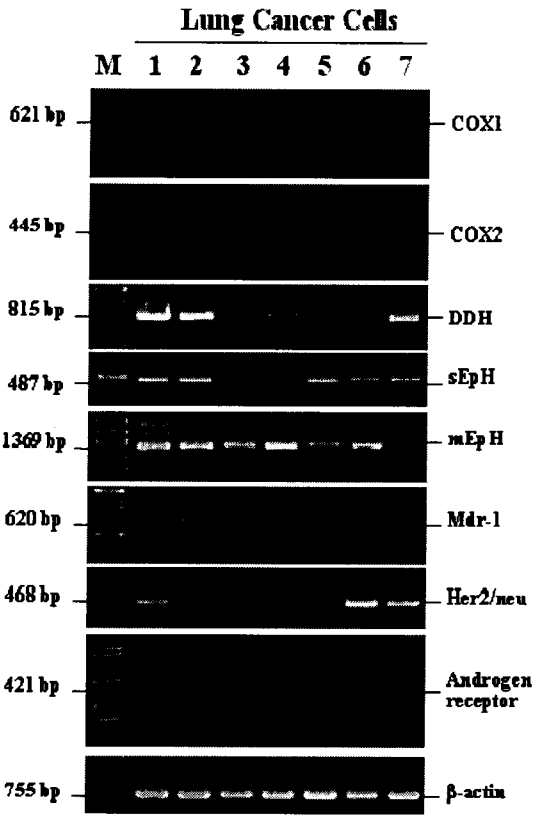

Figure 3 COX-2 gene expressions varied between squamous cell cancer cell lines and those of

adenocarcinoma origin (H125 and H226).

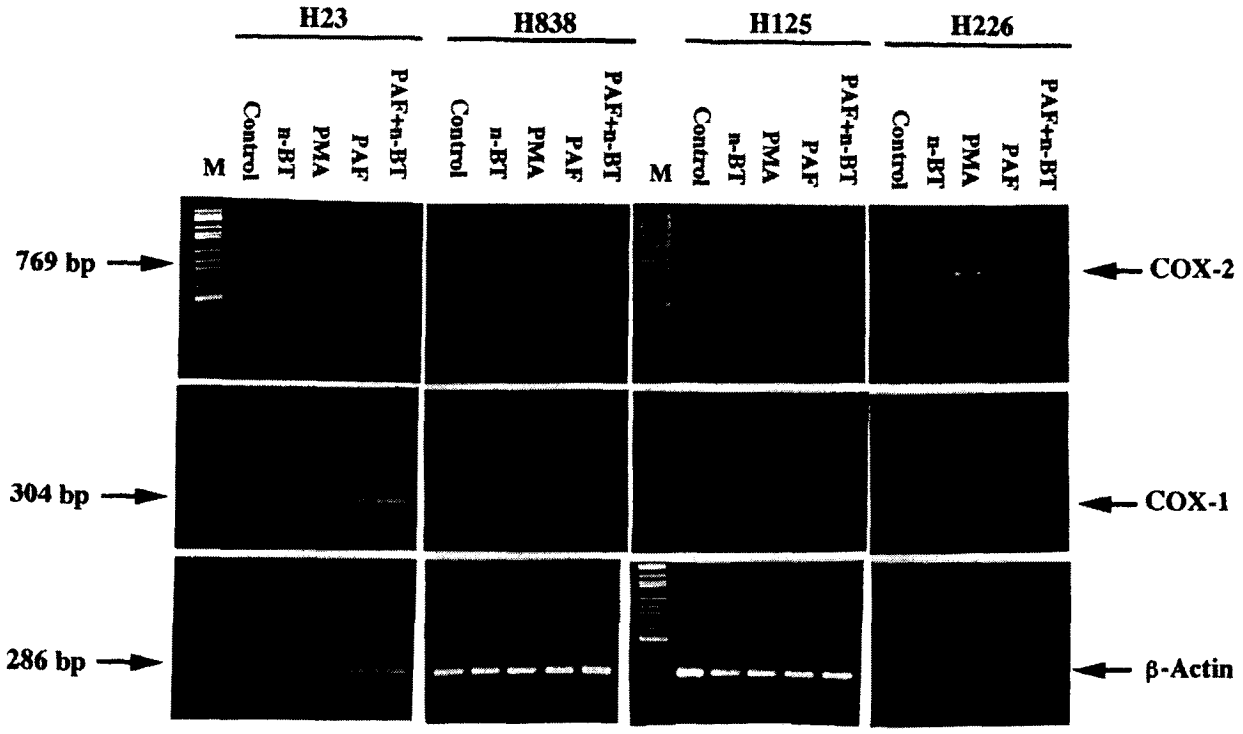

Table 1. Association of DDH Overexpression with Clinicopathological Parameters

Parameter	DDH expression		*P* value
	High (n = 317)	Low (n = 64)	
Age (yr) (n = 381)	63.4 ± 7.4	62.2 ± 6.8	0.177[a]
Gender			
Male (n = 304)	295	9	0.003[b]
Female (n = 77)	22	55	
Smoking			
Smokers (n = 275)	233	42	<0.001[b]
Non-smokers (n = 36)	14	22	
Mitotic index (#/10 HPF)	5.6 ± 3.7	4.7 ± 3.1	0. 03[a]
Number of Metastatic Lymph node			
N = 0 (n = 163)	-	-	
1 ≤ N ≤ 3 (n = 172)	134	38	<0.01[b]
N > 3 (n = 46)	42	4	
Stage			
I (n = 186)	135	51	<0.01[b]
II (n = 155)	142	13	
III (n = 35)	35	0	
IV (n = 5)	5	0	
Cell differentiation			
Well (n = 42)	33	9	0.052[b]
Moderate (n = 249)	115	40	
Poor (n = 90)	75	15	
Lymphovascular invasion			
Positive (n = 176)	143	23	0.016[b]
Negative (n = 205)	164	41	
Tumor recurrence			
Yes (n = 102)	90	12	<0.01[b]
No (n = 279)	227	52	
Biological Factors			
GST			
+ (n = 60)	17	43	<0.01[b]
- (n = 321)	300	21	
COX-2			
+ (n = 82)	70	12	0.072[b]
- (n = 299)	247	52	
nm23			
+ (n = 142)	90	52	<0.01[b]
- (n = 239)	227	12	
HER2/neu			
+ (n = 201)	177	24	<0.037[b]
- (n = 180)	140	40	
Mdr-1			
+ (n = 218)	165	53	<0.05[b]
- (n = 163)	152	11	

[a] Two-sided *P* value determined by *t* test

[b] *P* value determined by the χ^2 test

HPF: high power field