行政院國家科學委員會補助專題研究計畫成果報告

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二氫二醇去氫 °在乳癌的臨床意義以及其在乳癌癌細胞中的生理病理調控並與乙型環氧 °、抗藥性和 HER2/neu 基因之交互作用

Clinical significance of dihydrodiol dehydrogenase expression in breast cancer and the pathophysiological regulation of DDH in breast cancer cells and the interactions with COX-2, mdr-1 and HER2/neu gene expressions

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計畫類別：┆個別型計畫　　□整合型計畫

計畫編號：NSC89－2314－B－039－037－

執行期間： 89 **年** 08 **月** 01 **日至** 90 **年** 07 **月** 31 **日**

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國家衛生研究院與台北榮民總醫院合作病房

署立苗栗醫院 病理部

中　華　民　國 90 **年** 10 **月** 30 **日**

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一、中文摘要

本計畫是以研究二氫二醇去氫。(DDH) 與 Her2/neu 基因表現在乳癌的臨床意義為主題。以螢光原位基因偵測法 (FISH) 檢測 107 個乳癌病理切片，我們發現 36 位有 Her2/neu 基因放大的現象 (33.6%)。以免疫組織染色法，則發現有 49.5% (53/107) 的乳癌病人表現 DDH，而僅有 29.1% (21/72) 的乳癌之轉移淋巴結表現 DDH。第二型過氧化。 (COX2) 的表現卻有 57.9% (62/107)。COX2 的表現與 Her2/neu 基因放大以及 DDH 和 mdr-1 的表現成正比，但是與 PCNA、topoisomerase II 和 FHIT 的表現並無密切的關係。癌細胞中二氫二醇去氫。之高度表現者比低表現者其淋巴結轉移與腫瘤復發的機率都要高。而癌細胞中二氫二醇去氫。之高度表現者之抗藥性更顯著地比低表現者高出許多 (p = 0.002)。因此我們認為乳癌病人 DDH 的高度表現和腫瘤期別、淋巴血管轉移和抗藥性有關。

關鍵詞：二氫二醇去氫。、乳癌細胞、免疫組織化學染色、基因表現

ABSTRACT

In this study we investigated expressions of dihydrodiol dehydrogenase (DDH) and HER2/neu gene in patients with breast cancer. By fluorescent in situ hybridization (FISH), amplification of HER2/neu gene was detected in 36 patients. By using immunohistochemistry, we measured DDH expressions in 107 patients with breast cancer. Furthermore, expression of DDH was confirmed by immunoblotting and RT-PCR. Relation between DDH expression and clinical parameters was analyzed by statistical analysis. DDH overexpression was detected in 49.5% of pathological sections (53/107) and in 29.1% of metastatic lymph nodes (21/72). Interestingly, expression of cyclooxygenase 2 (COX2) was detected in 62 patients (57.9%), and COX2 expression was correlated with amplification of HER2/neu gene and DDH as well as mdr-1 expressions, but not related to PCNA, topoisomerase II or FHIT gene expressions. Compared with patients who had DDH overexpression in tumors, patients with low DDH expression had significantly lower incidence of lymph node metastasis and tumor recurrences. Interestingly, drug response rate was also significantly better in patients with low DDH expression than in those with DDH overexpression (p = 0.002). In conclusions, for patients with breast cancer, DDH overexpression was correlated with tumor stages, lymphovascular invasion, and poor drug response.

Keywords: dihydrodiol dehydrogenase, breast cancer, immunohistochemistry, gene expression, pathophysiological regulation

二、緣由與目的

Breast cancer is the second leading cause of cancer mortality worldwide among women. Most of the patients died were at the late stage of the disease when they were diagnosed.[1] However, some patients who were diagnosed at the early stage, and cared with the adequate surgical resection still died of cancer with the early recurrence and metastasis.[2] Breast cancer that metastasized beyond regional lymph nodes or has recurred after primary treatment (advanced breast cancer) becomes more incurable. Concomitant inflammation will even aggravate the seriousness of the disease.

Interestingly, epidemiological evidences showed that the use of nonsteroidal antiinflammatory drugs (NSAIDs), e.g., aspirin, indomethacin, and sulindac, could reduce not only the risk of breast cancers,[3] but also the risks of gastric, colorectal, and lung,[4] 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.[5]

In fact, beside cyclooxygenases, interconversion of prostanoids could be catalyzed by prostaglandin (PG) F synthase, a member of aldo-keto reductase family.[6] Recently, by using differential display to examine specimens of non-small cell lung cancer and lung cancer cell lines, we have identified overexpression of dihydrodiol dehydrogenase (DDH) that was not detected in the corresponding normal lung tissue.[7] DDH is also a member of aldo-keto reductases that catalyzes NADP-mediated oxidation of *trans*-dihydrodiols. In human liver, at least four isoforms of the enzyme (DDH1-DDH4) have been identified in the cytoplasm with monomeric mass of 36 kDa. Among these hepatic DDHs, DDH1 and DDH2 exhibit PGF synthase activity by converting PGD_2 into $9\alpha,11\beta$-PGF_2.[8] Detection of DDH overexpression in breast cancer cells would then provide an alternative link between chronic inflammation and carcinogenesis, and, possibly, the disease manifestation of breast cancer.

In this study, we used immunohistochemical method to determine the expressions of DDH and Her2/neu gene in surgical specimens from patients with breast. DDH expression in breast cancer was confirmed by 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 breast cancer were evaluated.

三、結果

As determined by immunohistochemistry, 53 patients (49.5%) were positive for DDH overexpression (Fig. 1), and DDH was also detected in 29.1% of metastatic lymph nodes (21/72). Expression of DDH was confirmed by immunoblotting and RT-PCR (Fig. 2A & 2B). By FISH, HER2/neu gene amplification was detected in 36 patients (Fig. 3). Interestingly, COX2 expression was detected in 62 patients (57.9%), and HER2/neu gene amplification was correlated with COX2, DDH and mdr-1 expressions, but not related to expressions of

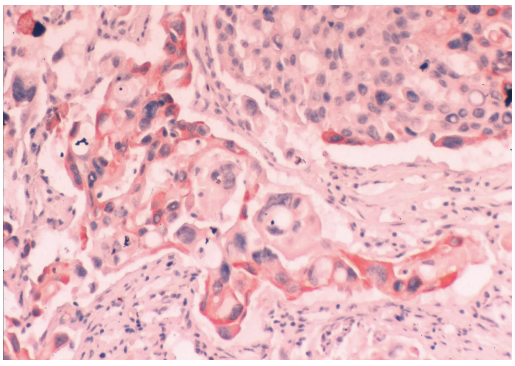

Fig. 1 Representative example of DDH expression in breast cancer cells detected by immunohistochemistry. DDH expression was not detected in the normal stroma (original

magnification × 200).

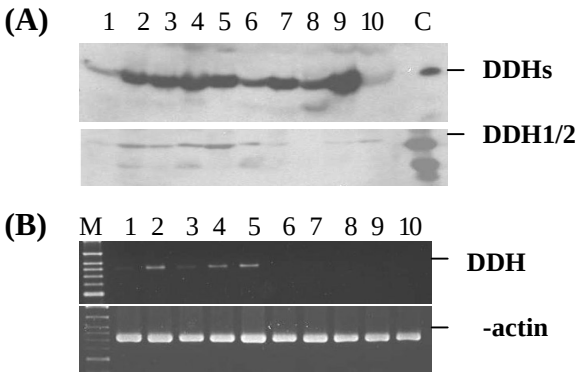

Fig. 2 Detection of DDH overexpression in breast cancer by (A) immunoblot analysis and (B) RT-PCR.

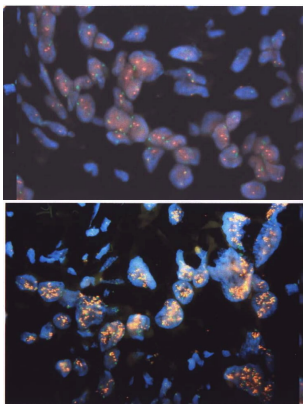

Fig. 3 Representative example of HER2/neu gene amplification in breast cancers detected by FISH method. In the upper panel, breast cancer cells have two normal copies of HER2/neu gene, and in the lower panel, breast cancer cells have amplification of HER2/neu gene (original magnification × 640).

PCNA, FHIT or topoisomerase II (Table 1). Nucleotide sequence of the DNA fragments from seven breast cancers matched with that of DDH3: XM_011858.1 Human aldo-keto reductase family 1, member C3 (DDH3) (AKR1C3), identities = 591/598 (98%).

四、討論

The results presented above demonstrate that gene amplification of HER2/neu gene in breast cancer correlated with overexpression of DDH, COX2 and mdr-1. Patients with DDH overexpression in breast cancer cells have significantly higher incidence of the early tumor recurrences that are frequently associated with the poor prognosis.

Table 1 Correlation of HER2/neu gene amplification with biological factors

	Copies of Her2/neu gene		
	2	3-5 or >5	*P*
COX2			
+	36	26	0.033
-	35	10	
DDH			
+	26	27	<0.001
-	45	9	
mdr-1			
+	32	26	0.008
-	39	10	
PCNA			
+	14	9	0.881
-	59	27	
FHIT			
+	37	14	0.196
-	34	22	
Topo II			
+	9	8	0.202
-	62	28	

Normally, DDH converts mutagenic PAH into catechol in the liver. Further oxidation of catechol could form PAH *o*-quinones that can rapidly conjugate with glutathione. However, DDH is not regularly expressed in the normal human breast. In addition to PAH metabolism, DDH could also be involved in drug detoxification. Shen *et al*[9] has shown that ethacrynic acid-induced drug-resistant human colon cancer cells could express high level of DDH. An elegant study by Ax *et al*[10] further demonstrated that anthracycline resistance in human stomach cancer cells could be mediated via DDH by altering daunorubicin into a less toxic daunorubicinol. However, intracellular events between DDH and drug function are yet to be elucidated. A variety of evidence suggests that DDH expression could be responsible for drug inactivation. In particular, chemical structures among anticancer drugs, e.g., adriamycin, VP-16 and mitoxantrone, and PAH-derivatives that are highly similar further indicate the possibility. Our results

showed not only the refractory mechanism of daunorubicin in breast cancer chemotherapy, but also the clinical association of DDH expression as a prognostic marker in breast cancer cells that correlated with disease progression and survival of patients with breast cancer.

五、計畫成果自評

1. **自從發現肺癌細胞高度表現自從發現肺癌細胞高度表現** DDH **後本實驗室更著手進行研究其他腫瘤；**
2. **不同腫瘤表現不同** DDH **的亞型；**
3. DDH1 **及** DDH2 **與抗藥性比較有關係；**
4. **此抗藥性與** mdr-1 **的表現無關。**

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