# 行政院國家科學委員會專題研究計畫　成果報告

## 二氫二醇去氫胸在皮膚癌細胞中的過度表現研究

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-039-025-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：中國醫藥大學醫學系

計畫主持人：吳孟澤

計畫參與人員：呂美寶

報告類型：精簡報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 11 月 2 日

## 一、 中英文摘要

Dihydrodiol dehydrogenase (DDH) is a member of the aldo-keto reductases (AKRs) superfamily which changes the aldehyde or ketone moiety to a corresponding alcohol by using NADH or NADPH as a cofactor. DDH is abundantly located in the cytoplasm of liver cells and normally converts mutagenic polycyclic aromatic hydrocarbons (PAHs) into catechol, indicating its important role in normal biological detoxification. Further oxidation of catechol could form PAH *o*-quinones that can rapidly conjugate with glutathione. Several recent reports had showed that DDH was associated with tumors in terms of disease progression and drug-resistance, indicating that DDH might play important roles in the carcinogenesis of various types of cancers. However, it remains largely unknown that by what mechanisms DDH might be involved.

Speculating that DDH might also be involved in tumorigenesis of certain type of skin cancers, we had done a series of preliminary experiments and revealed that DDH expression was increased in squamous cell carcinoma (SCC) but not in basal cell carcinoma (BCC) or melanoma, neither in normal skin cells (keratinocytes and fibroblasts). We hence hypothesize that DDH may also play an important role in the tumorigenesis SCC. Herein we propose to address this question by using retroviral transduction for DDH gene transfer to the SCC cells. DDH expression in transduced SCC will then be examined. More importantly, whether overexpression of DDH will alter certain aspects of tumor phenotypes/behaviors, including proliferation rate, invasion ability, drug-resistance and apoptosis-resistance will be determined. We hope that this project may help to address whether DDH may be importantly involve in skin cancer tumorigenesis.

二氫二醇去氫腖屬於醛酮還原腖家族(aldo-kero reductase)的一份子，而所謂的醛酮還原腖其功能則是藉由輔腖(如NADH或 NADPH)把化合物中屬於醛酮的部份轉化為醇類. 二氫二醇去氫腖廣氾的存在於生物體內的肝臟細胞，平時的功能主要可以把可致突變性的多環芳香碳氫化物(polyc aromatic hydrocarbons (PAHs) 轉化為二兒酚(catechol)，在生物體正常解毒過程中扮演一定的角色. 近來研究顯示二氫二醇去氫腖的表現與某些癌症的惡化與抗藥性有關，顯示二氫二醇去氫腖可能在癌症的病程發展中扮演重要的角色，然而其中機轉仍不明確.

我們因此懷疑二氫二醇去氫腖是否也與某些膚癌的病理機轉有關，而進行了先驅實驗. 結顯示二氫二醇去氫腖在鱗狀細胞癌細胞有明的表達，而基底細胞癌,黑色素細胞癌以及正皮膚細胞（角質細胞及纖維母細胞)則無表現據此我們假設二氫二醇去氫腖可能在鱗狀細癌病理機轉中扮演一定之角色. 為証實此假設我們於此提出本計劃，將利用基因轉介技術使氫二醇去氫腖於鱗狀細胞癌細胞中過度表達. 後我們將檢驗過度表達二氫二醇去氫腖之鱗細胞癌細胞,觀察這些經基因改造後的癌細胞控制組比較，各種癌細胞之形性與行為是否有差異. 我們預期此計劃將有助於進一步了解二二醇去氫腖於癌症病理機轉中所扮演之角色.

## 二、緣由與目的

Human dihydrodiol dehydrogenase (DDH) is a member of the aldo-keto reductases (AKRs) superfamily (Vogel, *et al* 1980), which changes the aldehyde or ketone moiety to a corresponding alcohol by using NADH or NADPH as a cofactor. DDH is abundantly located in the cytoplasm of liver cells as a monomeric (*M*r 34,000–36,000) protein (Flowers-Geary, *et al* 1993, Shou, *et al* 1992) and normally converts mutagenic polycyclic aromatic hydrocarbons (PAHs) into catechol (Shou, *et al* 1992, Vogel, *et al* 1980), indicating its important role in the constitutive detoxification process. Further oxidation of catechol could form PAH *o*-quinones that can rapidly conjugate with glutathione (Flowers-Geary, *et al* 1993, Glatt, *et al* 1979, Penning 1993)(Fig. 1). However, DDH is not ubiquitously expressed in other organs or tissues. Several recent reports had showed that DDH might play important roles in the carcinogenesis of various types of cancers including non-small cell lung cancer (NSCLC) (Hsu, *et al* 2001) and ovarian carcinoma (Deng, *et al* 2002). Hsu et al (Hsu, *et al* 2001) demonstrated that DDH was differentially over-expressed by NSCLC but not in the corresponding normal lung tissues. They further demonstrated that DDH expression may implicate cancer progression and serve as a prognostic marker for NSCLC(Hsu, *et al* 2001). Interestingly as well, increased expression of

DDH had been observed in cancer cells with chemotherapy-resistant phenotypes, including an ethacrynic acid-resistant human colon carcinoma cell line(Ciaccio, *et al* 1993) and a daunorubicin-resistant human stomach carcinoma cell line(Ax, *et al* 2000). Deng et al (Deng, *et al* 2002) recently further showed that increased expression of DDH may induce resistance to anti-cancer drug (Cisplatin) in human ovarian carcinoma cells. They firstly found that DDH was differentially expressed by a cisplatin-resistant variant of ovarian carcinoma over its cisplastin-sensitive parental cell line; furthermore, DDH gene transfection into cisplatin-sensitive ovarian carcinoma cells led to induction of cisplatin resistance, which was also associated with increased DDH enzyme activity(Deng, *et al* 2002). However, it remains largely unknown that by what mechanisms DDH might be involved in the tumorigenesis/drug resistance of these cancers, in spite of several theories being proposed. Deng et al [7] speculated that DDH's roles in detoxification of free radicals may lead to the apoptosis-resistance of cancer cells. Indeed, chemotherapeutic agents like cisplatin may increase the generation of reactive oxygen species in tumor cells resulting in an up-regulation of the apoptotic machinery [10], which might be de-activated by DDH's detoxification. Deng et al also found a decreased activation of caspase-3 (which may be manifestation of apoptosis resistance) in the cisplatin-resistant variant of ovarian carcinoma(Deng, *et al* 2002). Another possible mechanism for DDH to induce drug resistance, proposed by Hsu et al (Hsu, *et al* 2001), is the highly similar chemical structures between several anticancer drugs and PAH-derivatives, indicating that anticancer drugs may be subject to the enzyme activity of DDH.

The aforementioned evidences indicating that DDH may be involved in cancer progression prompted us to perform a series of preliminary experiments to examine whether DDH may also be involved in skin carcinogenesis. Several skin cancer cell lines (including melanoma, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)) were subject to real-time RT-PCR to measure the expression levels of DDH. Preliminary results revealed that DDH expression was increased in SCC but not in BCC or melanoma, neither in normal skin cells (keratinocytes and fibroblasts) . This result was further confirmed by western blotting experiments using DDH specific monoclonal antibodies to detect the expression of DDH in SCC at protein level.

Based on the above results, we hypothesize that DDH may also play an important roles in the tumorigenesis of certain types of skin cancers like SCC. Herein we propose to address this question by using retroviral transduction for DDH gene transfer to the SCC cells. DDH expression in transduced SCC will then be examined. More importantly, whether overexpression of DDH will alter certain aspects of tumor phenotypes/behaviors, including proliferation rate invasion ability, drug-resistance and apoptosis-resistance will be determined.

## 三. 結果與討論

### RNA interference of DDH expression by SCC cell line

To further understand the functional significance of DDH expression by SCC cells, we use vector-based RNA interference to knock down the expression of DDH by SCC cells (RNAi-SCC). As shown in Figure 1, after the transduction of DDH-interference vector, DDH expression was decreased to ~4.8-fold (by real-time PCR).

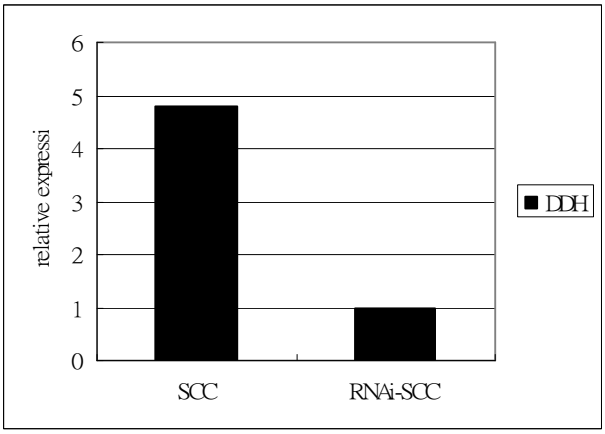



Figure 1. DDH expression by SCC and RNAi-SCC.

**Drug resistance of SCC cells correlates with DDH expression**

To determine whether DDH expression is related to drug resistance of SCC cells, parental SCC line and RNAi-SCC were subject to drug-resistance assay using cisplatin and MTT method. As shown in Figure 2, RNAi-SCC showed higher drug-resistance (represented by IC 50) compared with parental line (SCC). On the other hand, cisplatin-resistant SCC line (cis-r-SCC), established as previously described, showed decreased DDH expression down to ~4.7-fold compared to parental SCC line (Figure 3). Thus, DDH expression by SCC cells is conversely correlated with cisplatin-resistance.

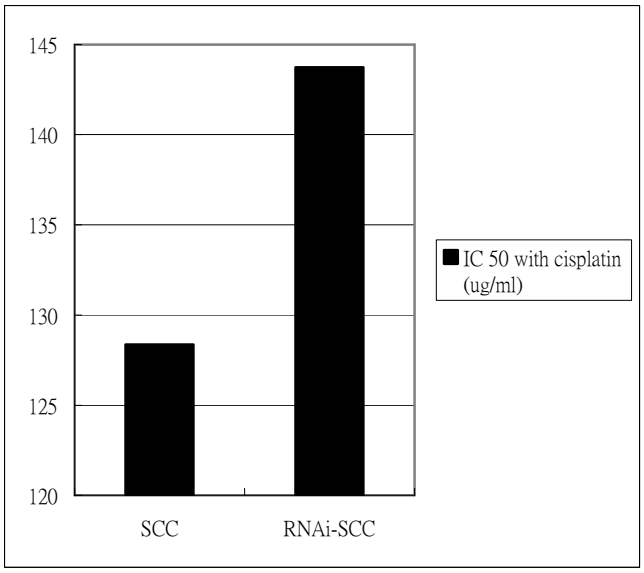



Figure 2. Cisplatin-resistance of SCC and RNAi-SCC cells.

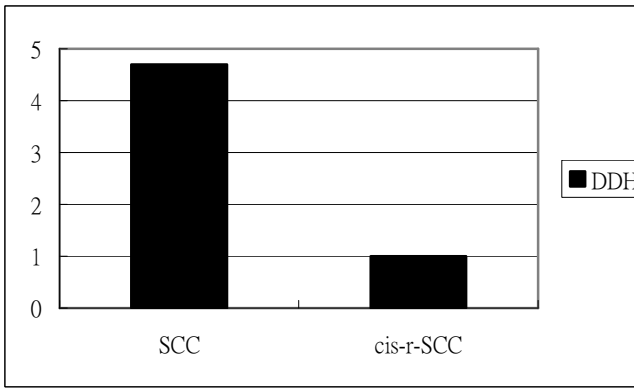
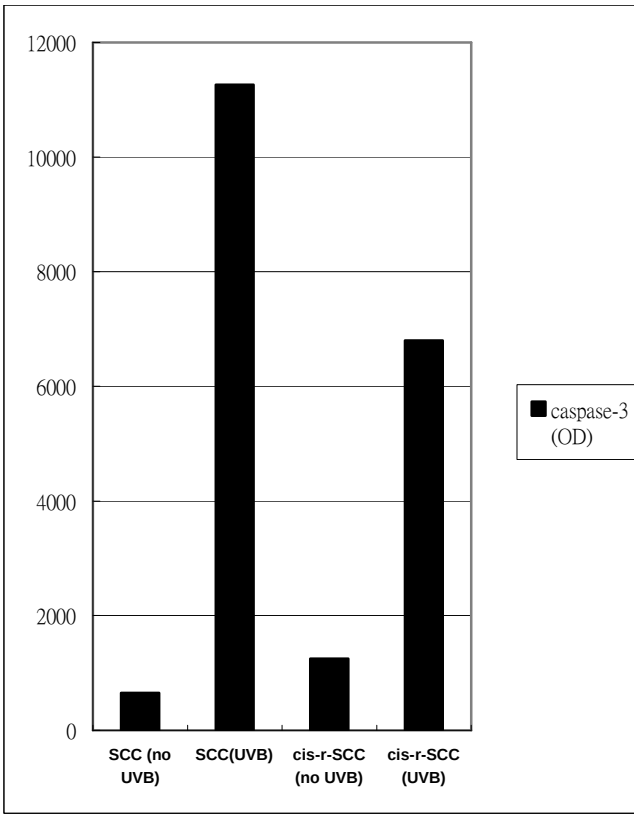



Figure 3. Relative DDH expression level of SCC and cis-r-SCC cells (by real-time PCR)

**UV-induced apoptosis of SCC cells and DDH expression**

To further explore whether DDH expression might be associated with other phenotypes of SCC cells, SCC parental line and RNAi-SCC cells were subject to UVB exposure for 24 hours, followed by detection of apoptosis reflected by caspase-3 level. As shown in Figure 4, there was more UVB-induced apoptosis manifested by parental SCC line compared to RNAi-SCC cells, indicating that DDH expression might also be conversely associated with apoptosis-resistance of cancer cells.



## 四. 成果自評

We have faithfully executed this granted project and are now preparing the manuscript for submission to scientific journal.

## 五. 參考文獻


Ax, W., Soldan, M., Koch, L. & Maser, E. (2000) Development of daunorubicin resistance in tumour cells by induction of carbonyl reduction. *Biochem Pharmacol,* **59,** 293-300.

Ciaccio, P.J., Stuart, J.E. & Tew, K.D. (1993) Overproduction of a 37.5-kDa cytosolic protein structurally related to prostaglandin F synthase in ethacrynic acid-resistant human colon cells. *Mol Pharmacol,* **43,** 845-853.

Deng, H.B., Parekh, H.K., Chow, K.C. & Simpkins, H. (2002) Increased expression of dihydrodiol dehydrogenase induces resistance to cisplatin in human ovarian carcinoma cells. *J Biol Chem,* **277,** 15035-15043.

Flowers-Geary, L., Harvey, R.G. & Penning, T.M. (1993) Cytotoxicity of polycyclic aromatic hydrocarbon o-quinones in rat and human hepatoma cells. *Chem Res Toxicol,* **6,** 252-260.

Glatt, H.R., Vogel, K., Bentley, P. & Oesch, F. (1979) Reduction of benzo(a)pyrene mutagenicity by dihydrodiol dehydrogenase. *Nature,* **277,** 319-320.

Hsu, N.Y., Ho, H.C., Chow, K.C., Lin, T.Y., Shih, C.S., Wang, L.S. & Tsai, C.M. (2001) Overexpression of dihydrodiol dehydrogenase as a prognostic marker of non-small cell lung cancer. *Cancer Res,* **61,** 2727-2731.

Penning, T.M. (1993) Dihydrodiol dehydrogenase and its role in polycyclic aromatic hydrocarbon metabolism. *Chem Biol Interact,* **89,** 1-34.

Shou, M., Harvey, R.G. & Penning, T.M. (1992) Contribution of dihydrodiol dehydrogenase to the metabolism of (+/-)-trans-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in fortified rat liver subcellular fractions. *Carcinogenesis,* **13,** 1575-1582.

Vogel, K., Bentley, P., Platt, K.L. & Oesch, F. (1980) Rat liver cytoplasmic dihydrodiol dehydrogenase. Purification to apparent homogeneity and properties. *J Biol Chem,* **255,** 9621-9625.