人類肝細胞癌侵襲、轉移的分子機轉

Molecular Mechanism of Invasion and Metastasis in Human Hepatocellular Carcinoma

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中文摘要。

吾人利用 Western blotting **分析並比較原發性與復發性肝癌病人檢體中之β**-catenin , connexin 43, connexin 32 and E-cadherin **表現。就原發性與復發性肝癌病人檢體而言，其肝癌部分之β**-catenin **與** connexin 43 **的表現量較非肝癌部分強，但是** connexin 32 **與** E-cadherin **的表現量則較弱。就肝癌部分而言，β**-catenin **與** connexin 43 **在復發性肝癌病人檢體的表現較原發性肝癌病人檢體強，其強度比值分別為** 1.6 **與** 2.2**。然而** connexin 32 **與** E-cadherin **的表現則無顯著差異。以上之結果顯示再復發肝癌病人檢體之β**-catenin **與** connexin 43 **的表現量的增加可能與病人再復發肝癌有關。**

關鍵字：復發性肝癌、β-catenin、connexin 43

I. ABSTRACT

The expression levels of β-catenin , connexin 43, connexin 32 and E-cadherin were performed by Western blotting. In primary and recurrent HCC cases, the expression level of β-catenin and connexin 43 in tumorous liver were higher than in non-tumorous liver. But the expression of connexin 32 and E-cadherin were decrease. In tumorous liver, there were significant difference of β-catenin, connexin 43 from recurrent HCC cases and those from primary HCC cases was compared, and the ratio was 1.6 and 2.2. Whereas no significant difference for connexin 32 and E-cadherin express level were observed between primary HCC and recurrent HCC cases. The results indicated β-catenin, connexin 43 may play important role in tumorous liver from patients with recurrent HCC.

Key words: recurrent HCC, β-catenin, connexin 43

II. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world, especially in sub-Saharan Africa and Southeast Asia (1,2). Since 1984, it is the leading cause of cancer deaths in Taiwan. About 6, 000-8,000 people died of this cancer every year in Taiwan. HCCs are characterized by the hypervascularity and have the propensities to invade portal veins, thus precluding many therapeutic strategies such as operation and transcatheter arterial embolization. It has been demonstrated that invasiveness of an HCC is the most crucial factor in determining the long-term outcome for the patient (3). Therefore, tumor angiogenesis, invasion and metastasis are the key point worthy investigation to further understand the tumor behavior and even therapy for HCCs.

Tumor invasion, metastasis and angiogenesis share a number of functional

similarities, and may be mutually stimulating. Invasion and metastasis of cancer is a multi-step process, involving the abilities of tumor cells to attach to the extracellular matrix (ECM) (adhesion), to degrade ECM component (proteolysis, and then to migrate through these ECM defects, and formation of new tumor vessels (angiogenesis)(4).

The attachment of cells to each other or to ECM is mediated by a number of adhesion molecules (such as cadherin and connexin). Connexin 32 (Cx 32) is the major gap junction protein in the liver (5). Cadherins (CD) are transmembranous Ca^{2+}-dependent cell-cell adhesion receptors that connect cells by hemophilic interaction (6). CDs are considered to play a major role in mediating intercellular adhesion. E-CD, one of the 4 subclasses of CD, is expressed in epithelial cells. Several in vivo studies of different types of human and mouse carcinomas have also shown a correlation between the expression of E-CD and the differentiation grade of these tumors (7). β-catenin is the major associate protein modulating the adhesion of cells through hemophilic interaction of CDs. Disruption or expression of truncated β-catenin lead to detachment of ectodermal cells in the embryo or loss of adhesion in human cancer cell lines (8). The interactions of β-catenin and CDs may be regulated in part through phosphorylation of β-catenin.

In the past 2 years, we investigated the expression of MMP-2 in tumorous liver from recurrent HCC cases were higher than in those from primary HCC cases. And the same result as the expression of MMP-9 and VEGF. In addition, CD 31 was performed to investigate formation of new tumor vessels by immunohistochemistry, which showed the density of new forming tumor vessels in tumor liver from recurrent HCC cases were much more than in those from primary HCC cases.

Since HCC is very common in Taiwan and the current therapeutic modalities are still unsatisfactory, studying the invasion and metastasis HCC will lead to a better understanding of tumor biology in this cancer, and hopefully may even shed a new light on its treatment.

II. MATERIAL and METHOD

A. Tissue samples. The matched pairs of tumor liver and non-tumor liver specimen were obtained from 18 HCC patients who had underwent curative hepatic resection for primary HCC, and after that resected recurrent HCC again past several months or years. Of these patients, 8 were women and 10 were men. As soon as HCC samples were removed, a part of the samples were frozen by liquid N_2 for extracting proteins, and another part were snap frozen in cooled in liquid N_2 in O. C. T. compounds for examining immunohistochemistry.

B. Western blotting analysis. For Western blotting, the HCC sample proteins were separated by a SDS-polyacrylamide gel electrophoresis, and then transferred into

nitrocellulose membrane. After blocked with 5 % nonfat milk in TBS, the membrane was incubated with diluted anti-mouse antibody against human β-catenin, connexin 43,connexin 32 and E-cadherin, separately. After washing, the diluted rabbit anti-sheep antibody in TBS was then incubated for 2 hours. And washes repeat, developing with alkaline phosphate NBT/ BCIP. The amount of β-catenin, connexin 43, connexin 32 and E-cadherin, separately, present are assessed using computer-assisted image analysis for measuring the level of band density.

C. Statistical analysis. All densitometry values are reported as mean ± SD. Statistical analysis is performed using t-test least significant difference, and p value <0.05 are considered significant.

III. RESULT

The expression of β-catenin, connexin 43, connexin 32 and E-cadherin

The expression levels of β-catenin , connexin 43, connexin 32 and E-cadherin were performed by Western blotting (fig1and table1). The results showed that the expression level of β-catenin and connexin 43 in tumor liver were higher than in non-tumor liver form primary HCC cases which were both significant different in statistic. The increment intensity of β-catenin, connexin 43 in tumor liver versus non-tumor liver from primary HCC and recurrent HCC cases was 1.7, 1.8, 3.1 and 5.3 fold, respectively. There were also significant difference of β-catenin, connexin 43 in tumorous liver from recurrent HCC cases and those from primary HCC cases was compared, and the ratio was 1.6 and 2.2.

The expression level of E-cadherin and connexin 32 in tumorous liver were decrease than in non-tumor liver from primary HCC cases which were both significantly different in statistic. The decrement intensity of E-cadherin and connexin 32 in tumor liver versus non-tumor liver from primary HCC and recurrent HCC cases was 0.5, 0.3, 0.4 and 0.2 fold, respectively. But there were no significant difference of E-cadherin and connexin 32 in tumor liver from recurrent HCC cases and those from primary HCC.

IV. REFERENCE

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