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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※組織蛋白脢 B, L 及 D 在人類復發性肝癌中扮演的角色 ※
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計畫類別：■個別型計畫　　□整合型計畫
計畫編號：NSC89－2314－B002－426
執行期間：89 **年** 8 **月** 1 **日至** 90 **年** 7 **月** 31 **日**

計畫主持人：李伯皇
共同主持人：

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執行單位：台大醫學院外科

中 華 民 國 90 **年** 10 **月** 31 **日**

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主持人：李伯皇　　執行機構及單位名稱 台大醫學院外科
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中文摘要

在亞洲及南非地區肝癌是最嚴重的癌症。然而高達 80**%的原發性肝癌病人於五年內會再併發復發性肝癌。就我們所知肝癌再復發的機制至今仍不十分清楚。組織蛋白酶(尤其是組織蛋白酶** B, L **及** D)**分布於人類的淋巴結、腎、脾、肝、肺、腦、腸胃組織、心及骨骼肌肉。有報告顯示當癌細胞藉由分解基片上的** collagen **及** elastin **而穿過基底膜以達成其分化及轉移目的的過程中組織蛋白酶扮演了很重要的角色。在此篇報告中我們利用** Western blotting **分析技術探討組織蛋白酶** B, L **及** D **在原發性及復發性肝癌病人檢體中的表現。結果顯示組織蛋白酶** L **在復發性肝癌中的表現明顯地較原發性肝癌強。當復發性肝癌病人的** tumor stage **較原發性肝癌嚴重時，組織蛋白酶** L **的表現反而較弱。其意義顯示組織蛋白酶** L **對於肝癌的再復發可能扮演一個重要的角色。**

Abstract

HCC is one of the most malignancies in Asia countries and South Africa. However, up to 80% of patients with primary HCC will recur again within five years. To our knowledge, the mechanism of HCC recurrence is still unclear. Cathepsins have been demonstrated in human lymph nodes, kidney, spleen, liver, lung, brain, gastrointestinal tissues, heart and skeletal muscle (5-7), especially Cathepsin B, L and D. Cathepsins were reported that played an important role in mediating the passage of malignant cells through the basement membrane by degrading collagen and elastin in the basal lamina resulting in invasion and metastasis. In the present study we investigated the expression of Cathepsin B, L and D in human patients with primary HCC and recurrent HCC by using of Western blotting. The result showed the expression of Cathepsin L in recurrent HCCs was significantly higher than those from primary HCCs in static. The tumor stage of recurrent HCCs was more advanced than of primary HCCs, the lower expression of the Cathepsin L was. It revealed that Cathepsin L may play an important role in human patients with HCC recurrence again.

Introduction

HCC is one of the most malignancies in Asia countries and in South Africa. Since 1984, it is the leading cause of cancer deaths in Taiwan. About 6000-8000 people died of this cancer every year in Taiwan (1). However, up to 80% of patients with primary HCC will recur again within five years. To our knowledge, the mechanism of HCC recurrence is still unclear.

The invasion and metastasis of malignant neoplasm progress through multistage processes: Cancer cells destroy the basement membrane, invade the extracellular matrix

(ECM), migrate to and invade the vessels, and lead to the formation of metastatic foci (2, 3). Several proteases appear to play a part in the metastasis of tumor cells. A correlation between metastatic potential and proteolytic activity has been demonstrated for collagenase, plasminogen activators of the urokinase-type, and Cathepsins (4). Cathepsins have been demonstrated in human lymph nodes, kidney, spleen, liver, lung, brain, gastrointestinal tissues, heart and skeletal muscle (5-7), especially Cathepsin B, L and D. Cathepsin B, L and D are lysosomal cysteine proteinase widely distributed in all eukaryotic and prokaryotic cells (8-14). It is widely known that many lysosomal enzyme activities are cleaved in malignant neoplasm (15).

Cathepsin B is the most abundant lysosomal cysteine proteinase in mammalian tissue (16), and found mainly within the lysosomes of normal tissues (17, 18). It has been suggested that Cathepsin B may degrade extracellular matrix and adhesion proteins, thus facilitating the invasion of host tissues by malignant cells (19-21). Cathepsin L has been suggested to promote oncogenic transformation and the malignant progression of several human neoplastic diseases including hepatocellular carcinoma (22-31). Furthermore, Leto *et al.* (32) have recently shown that the altered expression levels of Cathepsin L either at the cellular level or in body fluids be related to tumor progression as well to the onset of precancerous lesion in those nonneoplastic diseases which may undergo a malignant transformation. Cathepsin D was synthesized as the 43 kD preproCathepsin D, and which then processed into a active form, after further cleavage that yielded 28 kD and 15 kD form. Cathepsin D plasma levels seem to be independent of other tests or tumor marker routinely used in hepatology (33). In liver diseases, various workers (34-37) have elucidated the role of lysosomal enzymes as a cause of hepatocytic disorders. Growing experimental and clinical evidence indicates that lysosomal proteinases Cathepsin D, B and L may promote oncogenic transformation and tumor progression (38). Based on these above opinions, in the present study we investigated the expression of Cathepsin B, L and D in human patients with primary HCC and recurrent HCC by using of Western blotting. The result showed the expression of Cathepsin L in recurrent HCCs was significantly higher than those in primary HCCs in static. The tumor stage of recurrent HCCs was more advanced than of primary HCCs, the lower expression of the Cathepsin L was. It revealed that Cathepsin L may play an important role in human patients with HCC recurrence again.

Materials and Methods

A. Tissue samples

The matched pairs of tumor and non-tumor liver specimen were obtained from 14 HCC patients who had underwent curative hepatic resection for primary HCC, and recurrent HCC again several months after primary resection. Of these patients, 3 were women and 11

were men. All 14 HCC cases were divided into 3 groups according to UICC TNM staging. Group I was case no. 1~4, the tumor stage of recurrent HCCs was more advanced than those of primary HCCs. Group II was case no. 5 ~ 10, the tumor stage of primary HCCs was more advanced than those of recurrent HCCs. And the group III was case no. 11 ~ 14, the tumor stage of primary and recurrent HCCs were no difference. The clinical data were summarized of table 1. As soon as HCC samples were removed, the sample were frozen by liquid N_2 and stored at –80℃ until for protein extraction.

B. Western Blotting analysis

The expression of Cathepsin L, B and D were detected by Western blotting. The HCC 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 Cathepsin L, Cathepsin B and Cathepsin D at 4 ℃ overnight. After washing, the diluted rabbit anti-sheep antibody in TBS was then incubated for 2 hours. Wash repeatedly and developed with alkaline phosphate NBT/ BCIP. The amount band intensity of Cathepsin L, B and D were assayed using KODAK 1D Scientific Imaging System for measuring the intensity of band.

C. Statistical analysis

All the intensity of band values were reported as mean ± SD. Statistical analysis was performed using t-test, p value <0.05 were considered significant.

RESULT

A. The expression of Cathepsin L

The level of Cathepsin L in primary and recurrent HCCs were higher than those in non-tumor liver tissues, which were both significantly difference in statistics (fig 1). The increment intensity was 1.2 and 1.3 fold in primary and recurrent HCCs compared those in non- tumor liver tissues. Also the level of Cathepsin L in recurrent HCCs were higher than those in primary HCCs, which was significant difference and the increment intensity ratio was 1.2.

B. The expression of Cathepsin B

The levels of Cathepsin B in recurrent HCCs were increase 1.3 fold than those in non-tumor liver tissues, which were significant difference in statistics (fig 1). But no significant difference in Cathepsin B was observed between primary and recurrent HCCs.

C. The expression of Cathepsin D

The level of 43 kD preproCathepsin D in primary and recurrent HCCs were higher than those in non- tumor liver tissues, which were both significantly different in statistics (fig 1). The increment intensity in primary and recurrent HCCs versus those in non- tumor liver

tissues was 1.6 and 1.2 fold, respectively. Whereas no significant differences in 43 kD preproCathepsin D were examined between primary and recurrent HCCs. Fig1 demonstrated the expression of 28 kD Cathepsin D in HCCs were stronger than those in 43 kD preproCathepsin D, but the expression of 28 kD Cathepsin D in tumor and non- tumor liver tissues from all 14 primary and recurrent HCCs, showed no significant difference.

D. The correlation of Cathepsin L with the tumor stage of recurrent HCCs

For recognizing the correlation of Cathepsin L with the tumor stage of recurrent HCCs, all 14 HCC patients in this study were divided into 3 groups according to UICC TNM staging. Group I were case no. 1~4, the tumor stage of recurrent HCCs were more advanced than those of primary HCCs. Group II were case no. 5 ~ 10, the tumor stage of primary HCCs were more advanced than those of recurrent HCCs. And the group III was case no. 11 ~ 14, the tumor stage of primary and recurrent HCCs were no difference (table1). The intensity of Cathepsin L in recurrent HCCs compared primary HCCs was significantly higher only in the group II in static (fig 2).

E. The correlation of Cathepsin L with duration of recurrent HCCs

According to the duration of recurrent HCCs, the all 14 patients were divided into 2 groups, one group was within 12 month, and the other was over 12 month. Fig 3 showed the intensity of Cathepsin L of recurrent and primary HCCs both were no significant differences in each group.

Discussion

Cathepsins were reported that played an important role in malignant cell invasion and metastasis. In the present study we investigated the expression of Cathepsin B, L and D in human patients with primary HCC and recurrent HCC by using of Western blotting. Fig 1 demonstrated the expression of Cathepsin L and D (43 kD) in tumor liver tissues were higher than those in non-tumor liver tissues except Cathepsin B. The intensity of Cathepsin L in recurrent HCCs was significantly higher than that in primary HCCs. We suggested that Cathepsin L affected HCCs recurrence again than the other two. Furthermore, we examined the correlation of Cathepsin L with tumor stage of recurrent HCCs. Fig 2 showed the p value of group I (n=4) and II (n=6) was 0.06 and 0.02, respectively. So we inference that the tumor stage of recurrent HCCs was more advanced than that of primary HCCs, the lower expression of Cathepsin L was. As regards the correlation of Cathepsin L with duration of recurrent HCCs, fig 3 showed there was no relation between them.

In our study revealed that Cathepsin L may play an important role in human patients with HCC recurrence again.

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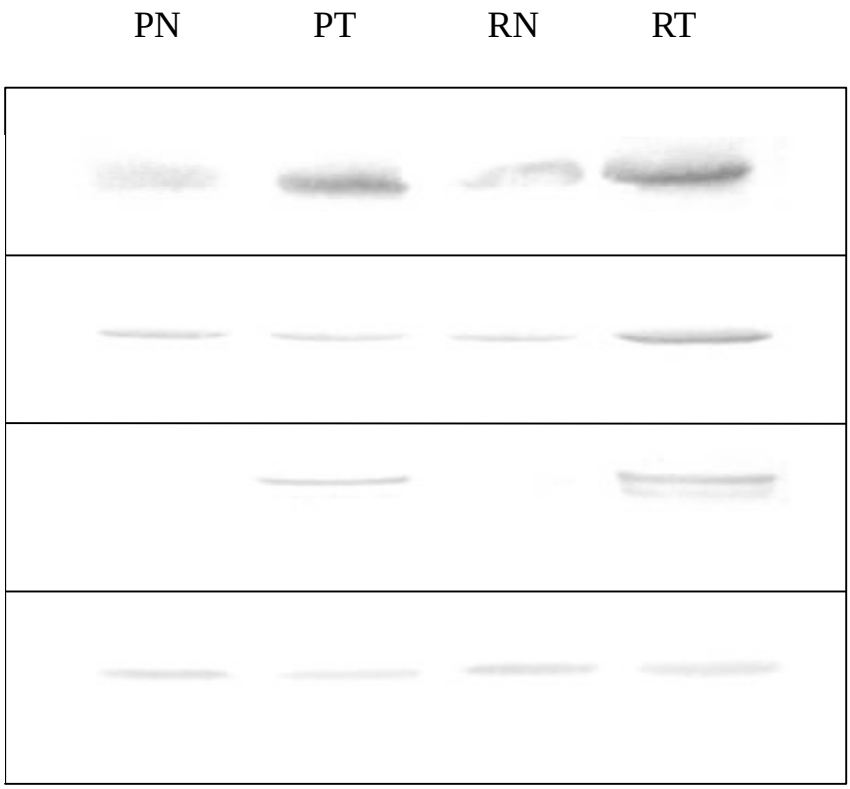
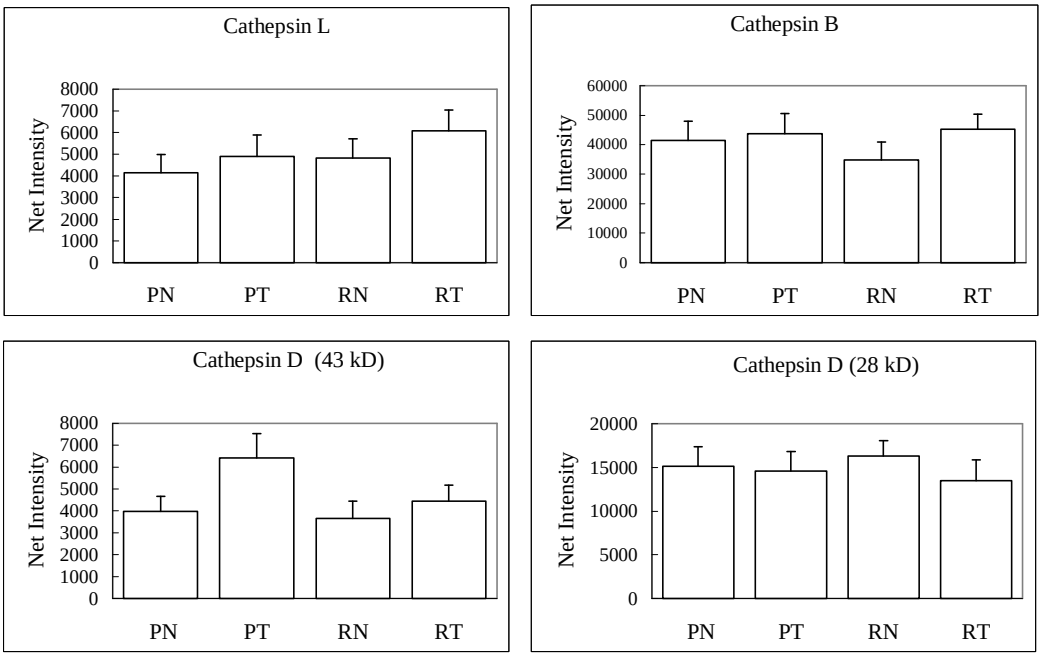
A.


PN
PT
RN
RT
Cathepsin L
Cathepsin B
Cathepsin D (43 kD)
Cathepsin D (28 kD)

B.


Cathepsin L
Net Intensity
8000
7000
6000
5000
4000
3000
2000
1000
0
PN
PT
RN
RT
Cathepsin B
Net Intensity
60000
50000
40000
30000
20000
10000
0
PN
PT
RN
RT
Cathepsin D (43 kD)
Net Intensity
8000
7000
6000
5000
4000
3000
2000
1000
0
PN
PT
RN
RT
Cathepsin D (28 kD)
Net Intensity
20000
15000
10000
5000
0
PN
PT
RN
RT

C

t test: p level

(* : p< 0.05; ns: no significant)

	Cathepsin L	Cathepsin B	Cathepsin D (43 kD)	Cathepsin D (28 kD)
PT vs. PN	*	ns	*	ns
RT vs. RN	*	*	*	ns
RT vs. PT	*	ns	ns	ns

Fig 1. The expression of Cathepsin L, B and D in human patients with HCC. The non-liver tumor tissues (PN and RN), primary HCCs (PT) and recurrent HCCs (RT) from 14 patients those were extracted proteins to electrophoresis on 12 % SDS-PAGE. After that Western blotting were performed to analyze the expression of Cathepsin L, B and D. A.: Western analysis of the expression of Cathepsin L, B and D. And which showed one of the 14 HCC patients. B: Semi quantitative analysis for measuring the net intensity level of band. All net intensity values were reported as mean ± SD. C: Statistical analysis was performed using t-test, p value <0.05 were considered significant.

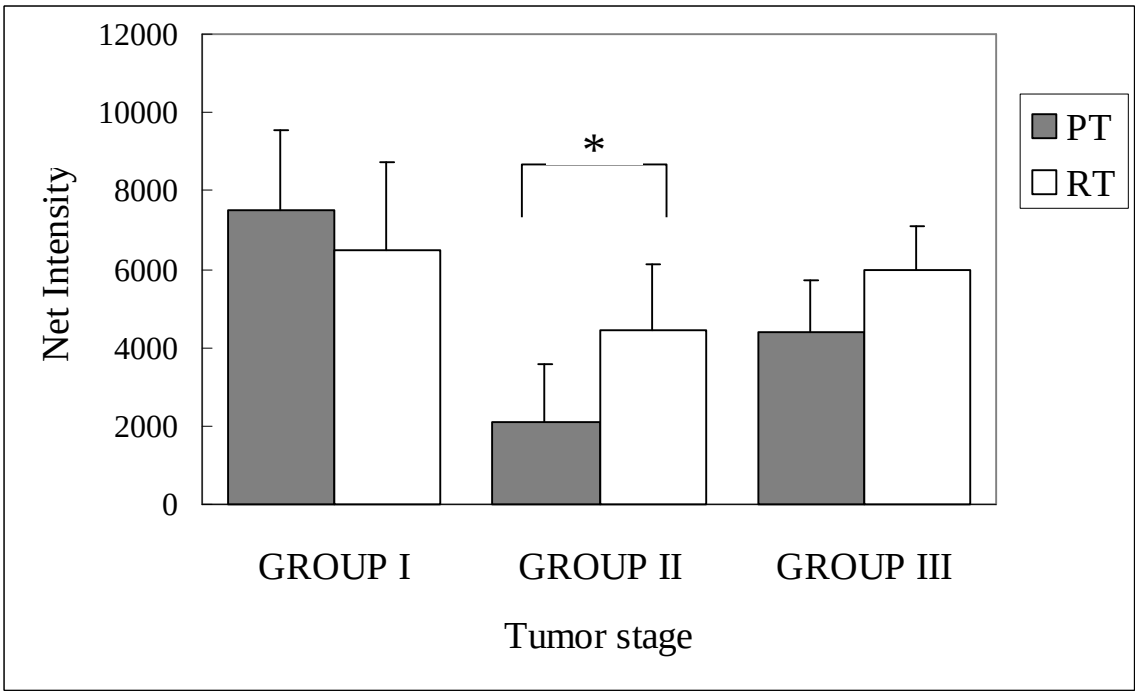


Fig 2. The correlation of Cathepsin L with the tumor stage of recurrent HCCs.
The tumor stage of recurrent HCCs was more advanced (group I), less advanced (group II) and no difference (group III) than that of primary HCCs. All net intensity values are reported as mean ± SD. (PT: primary HCCs, RT: recurrent HCCs. *: $p< 0.05$)

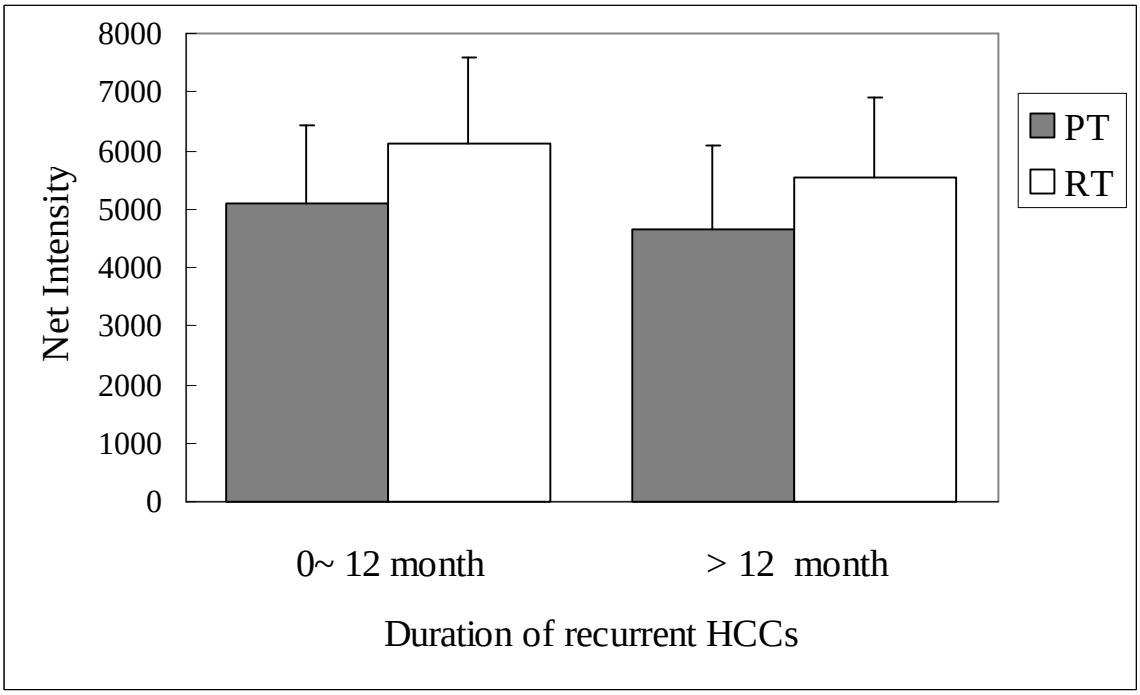


Fig3. The correlation of Cathepsin L with the duration of recurrent HCCs.
All net intensity values are reported as mean ± SD. (PT: primary HCCs, RT: recurrent HCCs.)