行政院國家科學委員會專題研究計畫 成果報告

以螢光二次元差異性電泳及質譜儀來分析肝癌栓塞治療副作用有關的血清蛋白質
研究成果報告(精簡版)

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中文摘要

關鍵詞：肝細胞癌，栓塞治療，蛋白質體學，二次元差異性電泳，質譜儀，血清

肝細胞癌（簡稱肝癌）是全世界常見的癌之一，自從 1984 至今，每年大約有 6000-8000 國人死於肝癌，雖然目前以超音波及甲種胎兒蛋白定期追蹤檢查，可以早期篩檢出肝癌，然而肝癌的治療仍不十分的理想。預後不良的原因之一，就是發現太晚。AASLD 最近的肝癌治療指引建議，對於無法做根除性治療的肝癌，栓塞治療是第一線的非根除性治療選擇。然而，栓塞治療最大的缺點就是副作用太大。對於栓塞治療的副作用，目前臨床上採用的方式是做症狀治療的支持性療法。不過若我們能對栓塞治療的分子機轉能有更進一步的了解，我們就有可能更有效的對抗栓塞治療所帶來的副作用。可惜的是，目前對於栓塞治療的副作用的分子機轉仍所知不多。

蛋白質體研究可以說是在後基因體時代的新型態研究工作。二次元電泳分析可以說是過去幾年蛋白質體研究的主流。雖然二次元電泳分析已被廣泛的應用在蛋白質體研究，然而沒有任何兩片的蛋白質膠體是完全一樣的，這使得不同膠片間與不同實驗室間的資料，很難做正確的比對。螢光二次元差異性電泳可以彌補上述的缺點。這個技術主要是奠基於使用Cy3與Cy5這兩種不同的螢光物，來標示要比較的兩種蛋白質體，然後將這兩種蛋白質體在同一片膠上跑電泳，然後再用電腦軟體來分析這兩種螢光的強度差異，用此來反應蛋白質表現量的差異。將有差異的點切出，以專一性蛋白酶水解之，再用質譜儀或胺基酸定序儀分析其胺基酸序列，即可判別原蛋白質的身份。由於這兩個蛋白質體是在同一片膠上跑電泳，所以分析時可以避開膠與膠之間的差異性，使得分析的結果較可靠，同時也可以做跨膠之間的比較。

過去兩年來我們已經成功的用 2D-DIGE 技術尋找出在肝癌組織與其相對應的非癌肝組織，蛋白質表現的差異。在目前的計畫中，我們用 2D-DIGE，來尋找肝癌病人接受栓塞治療之前及之後，血清中蛋白質表現的差異。我們共收集了 12 位肝癌病人栓塞治療之前及栓塞治療 4-7 天之後的血清，利用 2D-DIGE 的技術，我們發現有數個蛋白質，在栓塞後有明顯的表現增加，目前正在分析這些蛋白質的 ID 中。這些蛋白質就有潛力可能成為日後對抗栓塞治療副作用的生物標記。

英文摘要

Keywords: hepatocellular carcinoma, transarterial chemoembolization, Two-dimensional fluorescence difference gel electrophoresis, proteomics

Hepatocellular carcinoma (HCC) is one the most common malignancies in the world, especially in sub-Saharan Africa and Southeast Asia. Since 1984, it has been the leading cause of cancer death in Taiwan. About 8000 people died of this cancer every year in Taiwan. Despite the many treatment options, the prognosis of HCC remains dismal. A majority (70% to 85%) of patients present with advanced or unresectable disease. According to the guidelines of AASLD, transarterial chemoembolization (TACE) is recommended as first line non-curative therapy for non-surgical patients with large/multifocal HCC who do not have vascular invasion or extrahepatic spread. However, one of the downsides of TACE is the side effect.

The current practice in dealing with the side effects of TACE is the best supportive care. However, if we could know the specific molecules involved in the side effects of TACE, we might be able to give more specific therapy and improve the quality of life for HCC patients underwent TACE treatment. However, the underlying molecular mechanisms leading to the side effects of TACE are still not clearly known.

Two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) is a modification of traditional 2D technology and therefore can overcome some disadvantages of traditional 2D. In the past two years, we have successfully applied the 2D-DIGE to investigate the differentially-expressed proteins between HCC tissues and the corresponding non-tumor liver tissues. In this current project, we enrolled 12 HCC patients and collected the pre-TACE and post-TACE day 4-7 sera. We finished 2D-DIGE and found there were several proteins which were upregulated after TACE. We are now doing the in-gel digestion and protein Identification of these proteins.

If the proteins associated with side effects of TACE can be identified, we might further investigate whether these proteins can be used as the intervention targets. We hope that the side effects of TACE could be further reduced or even eliminated. If so, this could enhance the quality of life of HCC patients underwent TACE.

前言

Transarterial chemoembolization (TACE) treatment for hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is one the most common malignancies in the world, especially in sub-Saharan Africa and Southeast Asia. Since 1984, it has been the leading cause of cancer death in Taiwan. About 8000 people died of this cancer every year in Taiwan (1). Despite the many treatment options, the prognosis of HCC remains dismal. A majority (70% to 85%) of patients present with advanced or unresectable disease (2). According to the guidelines of AASLD, transarterial chemoembolization (TACE) is recommended as first line non-curative therapy for non-surgical patients with large/multifocal HCC who do not have vascular invasion or extrahepatic spread (3).

Side effects of TACE

However, one of the downsides of TACE is the side effect. TACE might induce the so-called post-embolization syndrome. This appears in more than 50% of the patients and consists of fever, abdominal pain and a moderate degree of ileus (3). Fever is a reflection of tumor necrosis, but a minority of patients may develop severe infectious complications such as hepatic abscess or sepsis (3-7). Though the post-embolization syndrome is usually self-limited in less than 48 hours and the patients can be discharged from the hospital (6), the side effects might be so severe that prolonged hospitalization is needed.

The current practice in dealing with the side effects of TACE is the best supportive care. However, if we could know the specific molecules involved in the side effects of TACE, we might be able to give more specific therapy and improve the quality of life for HCC patients underwent TACE treatment. It has been shown that interleukin-6 (8-10), interleukin-8 (8), macrophage-colony stimulating factor (11) are involved in the adverse reactions after TACE. However, the underlying molecular mechanisms leading to the side effects of TACE are still not clearly known.

Two-dimensional fluorescence difference gel electrophoresis (2D-DIGE)

To systematic search the molecules related to the side effects of TACE, we will use the proteomic approaches. In the past two years, we have successfully applied the 2D-DIGE to investigate the differentially-expressed proteins between HCC tissues and the corresponding non-tumor liver tissues (12). In this current project, we used the 2D-DIGE to systematically search the molecules related to the side effects of TACE.

研究目的

1. To successfully apply 2-D DIGE techniques for plasma samples
2. To identify the proteins with different expressions in the sera before and after TACE for HCC patients

研究方法

Patients

HCC patients who received TACE for HCC in National Taiwan University Hospital were enrolled. The clinicopathological data were retrospectively obtained from chart reviews. The serum samples were obtained immediate before TACE and 4-7 days after TACE.

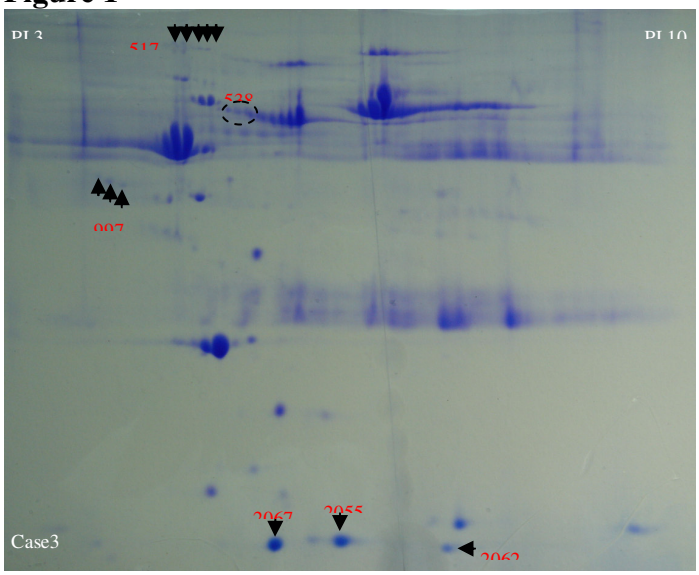
2D-DIGE

Serum albumin and immunoglobulin were removed by ProteoPrep Blue Albumin Depletion Kit (Sigma). The 2D-DIGE is done as described before (12). In short, 50 μg of protein sample was mixed with 400 pmol CyDye^{TM} (Amersham Biosciences). The Cy-dye labeled samples were separated by 2D-PAGE. Cy-dye labeled gels were visualized using a Typhoon^{TM} 9410 imager (Amersham Biosciences). Gel analysis was performed using DeCyder ^{TM} DIA (Difference In-gel Analysis) V5.0 (Amersham Biosciences). Intra-gel spot detection and inter-gel matching were performed using DIA V5.0 and BVA mode of DeCyder ^{TM} software (Amersham Biosciences) as described before (12).

結果與討論

A total of 12 HCC patients were enrolled. We finished 2D-DIGE and found several proteins were upregulated in the post TACE sera (figure 1). In-gel digestion and protein Identification of these proteins are ongoing now. We estimated that one more month is needed to complete the identification of these upregulated proteins. If the proteins associated with side effects of TACE can be identified, we might further investigate whether these proteins can be used as the intervention targets. We hope that the side effects of TACE could be further reduced or even eliminated. If so, this could enhance the quality of life of HCC patients underwent TACE.

Figure 1



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計畫成果自評

We collected the sera samples and finished 2D-DIGE as expected and scheduled. However, we are slightly behind our schedule in the protein identification. The reason is that the MS machine in our institute is dysfunction recently. This problem could be fixed and protein identification could be finished within one month.