行政院國家科學委員會
專題研究計畫成果報告

計畫編號：NSC 88-2314-B-002-324

題目：微小管與心臟衰竭

(Microtubule and Heart Failure)

執行期限：87 年 8 月 1 日至 88 年 7 月 31 日

執行機關：國立臺灣大學醫學院外科

主持人：朱樹勳

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一、 中文摘要

有實驗發現，細胞骨骼的變化似乎在高壓型心肌肥大上，伴演著重要的角色。本研究探討這類細胞骨骼變化，是否也發生於末期心臟衰竭之心肌。然而，初始的結果顯示似乎並非如此。為了對心臟衰竭有進一步的了解，我們應用蛋白質基因組分析的原則，對心臟蛋白質作概括性的研究。我們用二度膠質電泳，作擴張性心肌病變心肌蛋白質的分離，經由與正常心臟蛋白質之比較，可以找到與擴張性心肌病變有關的蛋白質。我們並使用質譜儀判斷甚麼蛋白質的表現，會隨擴張性心肌病變而變動。結果顯示，我們的方法，對解決這類複雜生物醫學課題將極有助益。

關鍵詞：心臟衰竭、蛋白質基因組分析、質譜儀、二度膠質電泳

Abstract

Some experiment demonstrated that cytoskeletal alteration plays an important role in contractile dysfunction of pressure overload cardiac hypertrophy. We investigated whether increased microtubule density was also associated with the end-stage heart failure. Unfortunately, there appeared to be no significant correlation between contractility and tubulin density. We have turned to proteomics approach to study how cardiac function is impaired in DCM hearts. We used 2-D electrophoresis to separate myocardial proteins and locate differentially expressed DCM-related proteins. LC/MS/MS method was used for identification of proteins that interest us. Our primary result indicates this may be a promising method to study complicated biomedical problems, for example, most of the cardiovascular diseases.

Keywords: Heart failure, proteomics, mass spectrometry, 2D gel electrophoresis

二、 源由與目的

Previous studies have demonstrated that cytoskeleton plays an important role in the contractile dysfunction of cardiac hypertrophy (for examples see Ref. 1-6), which is frequently associated with the end-stage dilated cardiomyopathy. Intriguingly, the cellular contractile dysfunction characteristic of pressure-

overload cardiac hypertrophy seems to be secondary to an abnormal microtubule function rather than due to aberrant myofilament activity. One of histological characteristics for these hearts is the increased microtubule density, which appears to result from both increased microtubule production (increased tubulin RNA) and enhanced microtubule stabilization (increased stable forms of microtubules) (7). The latter may result from an increase of microtubule-associated protein, like MAP-4 (8).

Eukaryotic microtubule polymer mainly consists of α- and β-tubulin monomeric subunits, as they carry out two essential functions in all higher eukaryotic cells (for reviews see Ref. 9-13). First, as the primary structural component of the mitotic spindle, microtubules are responsible for the segregation of replicated chromosomes into daughter cells at cell division. Second, in concert with actin filaments and intermediate filaments, microtubules play a major role in establishing and maintaining the dynamic spatial organization of the cytoplasm. Microtubules also function in specialized roles, comprising the major component of meiotic spindles and eukaryotic cilia and flagella, in establishment of the highly asymmetric morphology of neurons and serving as a substrate for the transport of vesicles and organelles within the cytoplasm.

It has been postulated that the increased microtubule density constitutes a viscous load on the contractile apparatus of cardiocytes (14). In end-stage dilated cardiomyopathy, interstitial fibrosis and cardiocyte hypertrophy are commonly seen. Chronic left ventricular failure may cause adaptive remodeling of the myocardium consisting of alterations in the geometry of the left ventricle and the orientation of the cardiac myocytes as well as disturbances in the biochemical function of the cellular organelles (15,16). Our project initially aimed to investigate whether increased microtubule density might play a role in myocardial failure. We have correlated the contractility parameter with the level of cytoskeletal proteins, like tubulins, in different cardiac segments. Our primary result revealed that there was no significant correlation between local contractility and tubulin density, which implies that dilated cardiomyopathy (DCM) at end stage, unlike pressure overload cardiac hypertrophy, does not associate with alteration of tubulin function. In order to study the mechanisms that participate in DCM progression, we decided to address this question using another approach, namely the proteome analysis. In this report, we summarize the progress of our experiments.

三、 結果與討論

Several groups have analyzed cardiac proteomes using two-dimensional gel electrophoresis system, but the common pitfall is the low turnout of myofibrile proteins. In order to analyze the proteins in myofilaments, we undertook differential extraction approach to separate myofibril proteins from regular soluble fraction. In the presence of high salts and detergent, most of cardiac proteins became solubilized, while a small portion of myocardial proteins remained insoluble. Their composition is similar to previously isolated myofibril fraction (Fig. 1).

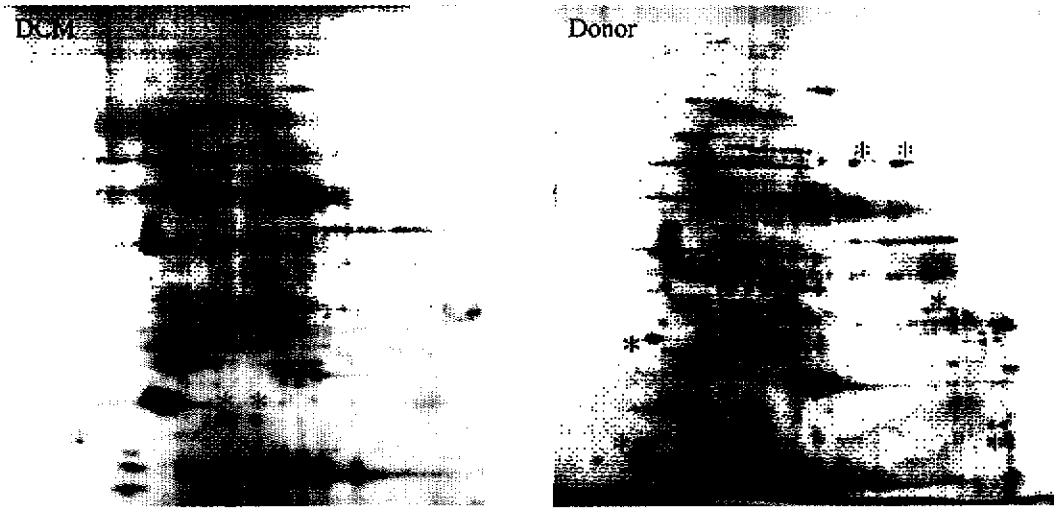

Figure 2. Differential protein expression in soluble fraction between DCM and donor hearts. The protein components in the soluble fraction were first separated in an IEF gel and then resolved by SDS-PAGE analysis. The gel was developed by silver stain method and the stars (*) denoted those protein spots whose expression is varied in DCM hearts versus donor hearts.

We then separated cardiac proteins in a 2-D gel system, which has IEF as the first dimension and SDS-PAGE as the second dimension. Both fractions were analyzed respectively, as illustrated in Fig. 2 and Fig.3. The proteins in soluble fraction are quite similar to what other groups have observed (Fig. 2). More importantly, we found that several proteins were differentially expressed in DCM myocardium. On the other hand, the particulate fraction was also subjected to 2-D gel analysis and the protein pattern is quite distinct from that derived from the soluble fraction (Fig. 3). Several major spots in the gel were first digested with trypsin, and then analyzed by LC/MS/MS experiment for protein identification. We found that this fraction indeed contained most of the myofibril proteins, including myosin light chains, myosin

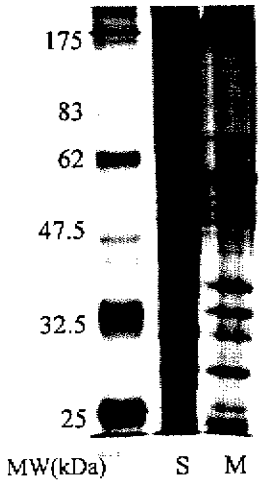

Figure 1. Soluble fraction and myofibril fraction by differential extraction. Myocardial specimen was homogenized in a buffer containing 0.2% Triton X-100 and 400 mM NaCl. The homogenate was separated into two fractions by high-speed centrifugation, which included soluble and particular fractions. The proteins in particular fraction was extracted in 2X SDS sample buffer at 100°C. Both soluble and particulate fractions were concentrated using methanol-chlorofrom method, and then were resuspended in urea lysis buffer. After adding 6X SAS sample buffer, we analyzed the protein components on an 11% polyacrylamide gel and developed the gel by silver stain method.

heavy chain, actin, tropomyosin, and etc. Several proteins in the particulate fraction were also differentially expressed in DCM myocardium. This demonstrates that our procedure will help us to

identify DCM-associated proteins.

四、 計畫成果自評

Based on our data, we found that cytoskeleton change was probably not related to myocardial contractility. In order to further explore the mechanisms that elicit cardiac malfunction in DCM hearts, we decide to address this issue using the proteomics approach. Our primary results indicate that our approach is sufficient to screen out DCM-associated proteins. We particularly emphasize on the analysis of myofibril proteins, since their activities are pertinent to cardiac function. Therefore, we will continue to use differential extraction method to collect sufficient amounts of myofibril proteins. We also demonstrate that the protein separated by 2-D gel electrophoresis is suitable for protein identification by LC/MS/MS experiment.

We will employ this approach to do more detailed analysis on cardiac proteins. We will test whether altered expression of certain DCM-associated proteins is specifically found in DCM hearts as well as to test whether their altered expression is related to the progression of DCM. Using this approach, we hope to identify certain protein markers that help us in diagnosis or treatment of DCM patients clinically.

This pilot study also give us a good opportunity to evaluate our procedures, which may be very useful in the studies of other cardiovascular diseases. Most of cardiovascular diseases are very complex and we frequently need better strategies to solve this kind of biomedical problems. We think the proteomics approach is very useful for the research on cardiovascular diseases.

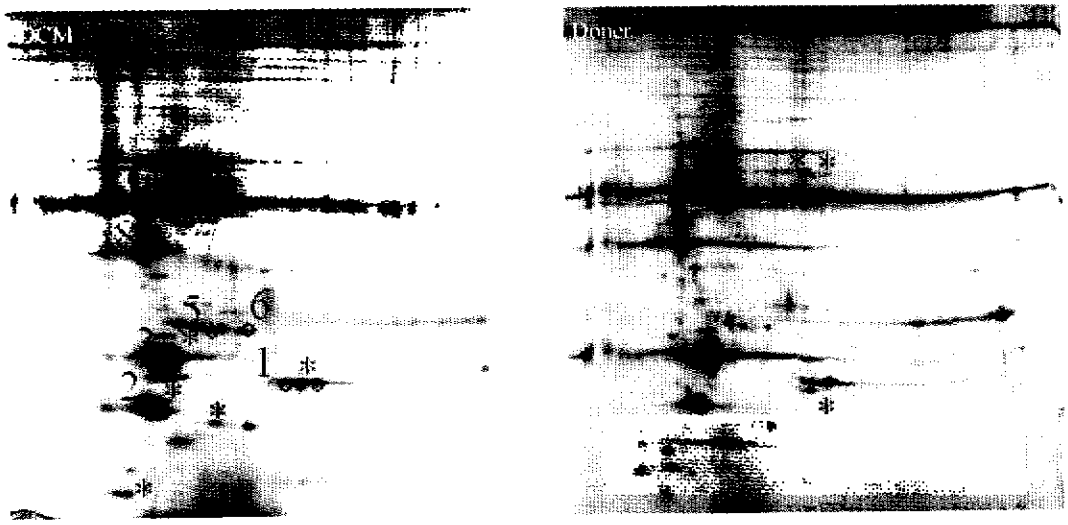

Figure 3. Differential protein expression in the particulate fraction between DCM and donor hearts. The particulate fractions were analyzed by the 2-D gel system. The proteins with varied expression were marked by the star (*). Several proteins were identified using LC/MS/MS method, including αB crystallin (1), myosin light chain 2 (2), myosin light chain 3 (3), actin (4), HSP-27 (5), ubiquinol-cytochrome C reductase (6), troponin T (7) and tropomyosin α (8).

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