行政院國家科學委員會專題研究計畫　成果報告

蛋白質精胺酸甲基化之蛋白體分析

計畫類別：個別型計畫
計畫編號：NSC91-3112-B-040-001-
執行期間：91 年 05 月 01 日至 92 年 04 月 30 日
執行單位：中山醫學大學生命科學系

計畫主持人：李娟

報告類型：完整報告

處理方式：本計畫可公開查詢

中　華　民　國 92 年 7 月 18 日

行政院國家科學委員會專題研究計畫成果報告

蛋白質精胺酸甲基化之蛋白體分析

Proteomic analysis of protein arginine methylation

計畫編號：NSC 91-3112-B-040-001

執行期限：九十一年五月一日至 九十二年四月三十日

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一、中文摘要

後基因體時代的一個主要的挑戰就是研究細胞中全部表達出來的蛋白質—也就是蛋白體。轉譯後之修飾使蛋白質的複雜度提昇，不只僅是二十個氨基酸之排列組合而已。本計劃就是要用蛋白體的研究方法來分析一種轉譯後的修飾—蛋白質精胺酸甲基化，主要為使用蛋白質體的方法二維電泳後以質譜來分析。我們也已成功的找到一些已知的甲基接受蛋白和某些可能的新甲基接受蛋白。此計劃我們致力於以下四個方向：（一）蛋白質體法整體性的分析甲基接受蛋白。（二）分析所有蛋白質精胺酸甲基接受者在細胞中分佈的情形。（三）探討透過上述的方法找出之新甲基接受蛋白質的特性。（四）我們將藉由蛋白質體的研究方法法來分析不同生物醫學樣本的精胺酸甲基接受蛋白，以找出蛋白質精胺酸甲基化和人類特殊疾病之間的相關性。透過這些蛋白質體的研究我們將可明確得知甲基接受蛋白精胺酸甲基化的功能，使我們能更加瞭解此種轉譯後修飾所扮演的的角色。

關鍵詞: 蛋白體，蛋白質精胺酸甲基化，轉譯後修飾

Abstract

In the post-genomic era the major challenge is to investigate the complete expressed proteins in cells, the proteome. Posttranslational modifications increase the complexity of proteins beyond the combination of twenty amino acids. The project will investigate one type of protein posttranslational modification— protein arginine methylation using the proteomic tools, basically two dimensional gel electrophoresis followed by mass spectrometry to identify specific protein spot. We have started the investigation of the methylaccepting proteins using proteomic approach and successfully identified some known and novel putative methylaccepting proteins. In this project we focus on four areas. Firstly, we perform global analysis of protein methylaccepting proteins by the proteomic approach. Secondly, we analyze protein arginine methylacceptors globally in subcellular localization. Thirdly, we characterize the novel protein methyl- acceptors identified through the above approaches. At last, we

analyze the relationship of protein arginine methyl- -ation with specific human diseases by proteomic analyses of the arginine methylaccepting proteins in different biomedical samples. Through these studies we will identify, and specify the function of arginine methylation of the methylaccepting proteins through the proteomic approach, to help to better understand the roles of this posttranslational modification.

Keywords: proteomics, arginine methylation, posttranslational modification

二、緣由與目的

In the post-genomic era the major challenge is to investigate the complete expressed proteins in cells, the proteome. Posttranslational modifications increase the complexity of proteins beyond the combination of twenty amino acids. Protein arginine methylation is an irreversible modification on the guanidino nitrogen of the arginyl residues that accounts for the majority of stable protein methylation events in cells. The modification is likely to be involved in various cellular functions such as signal transduction, protein subcellular localization, and transcriptional regulation (McBride et al., 2001). Since the identification of the first protein arginine methyltransferase (PRMT) gene PRMT1 (Lin et al., 1996), by now six PRMT genes have been identified in the mammalian system. Why in the mammalian system so many different methyltransferases are required to modulate different biological functions, how the methyltransferases are evolved and whether the methyltransferases has different substrate sets are interesting questions.

Thus we focus on four different areas in order to identify, and specify the function of arginine methylation of the methyl-accepting proteins through the proteomic approach, to help to better understand the roles of this posttranslational modification.

1. *To perform global analysis of protein methylaccepting proteins by proteomic approach*: From previous analyses we found that most of the methylaccepting proteins are low abundant proteins (Huang et al., 2002), difficult for following identification by mass spectrometry. In cooperation with the Proteomic Core, we will enrich the methylaccepting proteins by different separation strategies and then analyze the mass spec data to identify more methylaccepting proteins.
2. *To characterize the novel protein methylacceptors identified through above approaches:* We are analyzing prohibitin, a putative methyl-accepting identified through our proteomic approaches, and an RGG –box containing protein found by Blast search, whether they are real methylacceptor.
3. *To analyze globally protein arginine methylacceptors in subcellular localization:* Subcellular localization of hnRNP A2/B1 and other hnRNP in yeast had been shown to be affected by their arginine methylation status. We thus would like to use the proteomic approach to systematically analyze the distribution of methylaccepting proteins in subcellular fractions. The results will help to interpret the physiological function of this modification.
4. *To analyze the relationship of protein arginine methylation with specific human diseases by proteomic analyses of the*

arginine methylaccepting proteins in different biomedical samples: Abnormal protein methylation may be involved the development of certain human diseases such as cancer, autoimmune disease and some genetic diseases, for example, fragile X mental retardation and spinal muscular atrophy. Whether the unbalanced distribution of arginine methylation might lead to certain disease is an interesting and important question.

三、結果與討論

For the proposed specific aims, firstly we set up the IPGphore IEF system in our laboratory and have tried the conditions of analyzing samples using narrow pH-range IPG strips. Secondly, we have started to examine the stable protein methylation and the expression of arginine methyltransferases in different cell lines under various specific treatments. Specifically, stable protein methylation in Hela cells were analyzed. AdOx treatment together with a translation inhibitor cycloheximide inhibits the appearance of methylacceptor upon AdOx treatment –suggesting that AdOx accumulate methylaccepting proteins by blocking modification on newly synthesized proteins. We are analyzing the methylaccepting sites on fibrillarin, a standard RGG containing methylaccepting protein for setting up the standard protocol for analyzing the methylation sites in the protein.

Thirdly, we had investigated the subcellular localization of various methyl-accepting proteins in response to the methylation status. Western blot analyses of the subcellular fractions from lymphoblastoid cells and immunohisto-chemical studies of Hela cells treated with AdOx or not had been performed. We had looked at the expression of FMRP, FXR2, hnRNPA1, hnRNPA2/B1, Sam 68, PRMT1 and the mono- and di-methylarginine species by a specific Ab. However, no convincing differences could be identified.

As for novel methylaccepting proteins, we are subcloning prohibitin, a putative methylaccepting protein identified through our previous investigation, into a bacterial expression vector. We have also successfully subcloned a novel RGG containing protein identified through BLAST search and are examining the possibility of its being a arginine methyl-acceptor. By now six different arginine methyltransferase genes have been identified in the mammalian system. For future more detail analyses, investigation of the enzyme-substrate specificity will be crucial. We are trying to complete our collection of the methyltransferase clones as well as continuous search for putative new arginine methyltransferase genes in the database. We started bioinformatic studies of the methyltransferases in different model systems for clues of the evolutions of the modification systems.

四、計畫成果自評

For the first year, due to the unusual starting time of the project and the delay of

the final approval of the money from NSC, we had some difficulties in the first 2-3 months starting the studies. We had problems recruiting research assistants until July after the graduation seasons. Anyway we had the systems set up in the past few months. As for Mass analyses, we now have good communications with the Core facilities, and with their cooperation we are more likely to obtain the ideal data for posttranslational modification analyses. We are now preparing four-five manuscripts based on the research of the first year.

五、參考文獻

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執行單位：中山醫學大學生命科學系

中　華　民　國　九十二　年　七　　月　十八　　日