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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□赴國外出差或研習心得報告一份

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□出席國際學術會議心得報告及發表之論文各一份

□國際合作研究計畫國外研究報告書一份

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行政院國家科學委員會專題研究計畫成果報告

第四型黏多醣症之突變分析

Mutation Analysis of Mucopolysaccharidosis Type IV

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一、 Chinese Abstract

黏多醣症是一群罕見之遺傳代謝疾病，一共分為六型。病患體內由於分解黏多醣酵素之基因缺陷，使得分解黏多醣所需之特定醣酵喪失功能，造成異常黏多醣日漸堆積在骨骼和器官中。患者因而產生生長遲滯、智力障礙、骨骼異常，患者常因呼吸道感染而致命。據估計國內約有一百五十個至兩百個黏多醣症病例，其中以第二型最為普遍，第三型次之，第四型為第三普遍之黏多醣症。第四型黏多醣症亦因缺陷之黏多醣酵素之不同，分為兩亞型—四 A 型及四 B 型。有關黏多醣症基因突變之研究，國外已相當進步，國內則起步較晚，目前已知有師範大學生物系李桂楨教授致力於第一型及及第三型黏多醣症突變機制之釐清，原馬偕醫院張建國醫師致力於第二型黏多醣症突變機制之釐清。本單位受台灣黏多醣症協會之委託，特提出此一年期計畫，研究第四型黏多醣症之基因突變機制。

本計畫之研究要點如下：

1. **藉由台灣黏多醣症協會之協助，建立第四型黏多醣症病人之淋巴球母細胞株及/或皮膚纖維母細胞株。**
2. **藉由酵素測定，將第四型黏多醣症病人區分為第四 A 型及第四 B 型，以利之後之突變分析。**
3. **第四 A 型黏多醣症之突變分析：利用聚合酶鏈反應複製第四 A 型黏多醣症之致病基因﹝GALNS﹞之十四個 EXON，藉由單股多型性分析，篩檢出異常之核酸片段，進行直接核酸定序分析以找出突變點。**
4. **第四 B 型黏多醣症之突變分析：自已建立之第四 B 型黏多醣症病人淋巴母細胞株及/或皮膚纖維母細胞株純化核醣核酸，再藉由反轉錄—聚合酶鏈反應﹝RT-PCR﹞複製第四 B 型黏多醣症致病基因﹝GLB1﹞之互補去氧核醣核酸﹝cDNA﹞，再以直接核酸定序分析，找出 GLB1 互補去氧核醣核酸上之核甘酸改變。**

利用定點突變﹝site-specific mutagenesis﹞及過渡轉殖﹝transient transfection﹞，將帶有已知突變之 GALNS 或 GLB1 之全長互補去氧核醣核酸，經由 pcDNA3 哺乳細胞表現載體，轉殖入 COS-7 細胞，或第四型黏多醣症病人之纖維母細胞，以研究其突變與功能之關係。

關鍵詞：黏多醣症、淋巴球母細胞株、皮膚纖維母細胞株、聚合酶鏈反應、單股多型性分析、直接核酸定序分析、反轉錄—聚合酶鏈反應﹝RT-PCR﹞、互補去氧核醣核酸﹝cDNA﹞、定點突變﹝site-specific mutagenesis﹞、過渡轉殖﹝transient transfection﹞

二、English Abstract

The mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by the defuiency of lysosomal enzymes that catalyze the stepwise defradationof glycosaminoglycans (mucoppolysaccharide). The accumulation of various glycosaminoglycan molecules would

eventually results in cell, tissue, and organ dysfunction. There are ten known enzymes involved MPS, which were divided into six types depending on the majopr clinical symptoms. In Taiwan, it was estimated that the total number of MPS patients is between 150 to 200. MPS II is the most popular, MPS III next, and MPS IV is the third most common. MPS IV is divided into two subtypes: 4A and 4B, depending on the enzymes involved. In the past three years, Dr. Lee-Chen of National Norman University had been devoted into the mutation analysis of MPS I and MPS III and Dr. Chang had been studying the mutation causes of MPS II. We, as requested by the Taiwan MPS Society's request, try to investigate the mutation causes of MPS IV. The main tasks of this proposal are listed below:

1. Through the help of Taiwan MPS Society's help, we will establish the lymphoblastoid cell lines and/or skin fibroblast cell lines.
2. We will divide the MPS IV patients into MPS IVA and MPS IVB by use of enzyme assays.
3. Mutation analysis of MPS IVA were performed by PCR amplification of 14 exons of GALNS the MPS IVA coding gene. The PCR fragments were screened by single strand conformation polymorphism (SSCP) analysis and fragments that exhibit irregular shifts in SSCP were subjected to direct DNA sequencing to find out the nucleotide change(s) that cause the shifts.
4. Mutation analysis of MPS IVB was performed by RT-PCR amplification of total RNA prepared from those MPS IVB patients' lymphoblast cells and/or skin fibroblast cells. Direct DNA sequencing will be performed to identify the nucleotide change(s).
5. The identified mutation will be transferred to the normal cDNA by site-specific mutagenesis and cloned in the pcDNA3 mammalian expression vector, which will be transient transfected into COS-7 and/or MPS IV cells to study the mutation mechanism.

Keywords：mucopolysaccharidoses (MPS), lymphoblastoid cell lines, skin fibroblast cell lines, PCR, strand conformation polymorphism (SSCP) analysis, Direct DNA sequencing, RT-PCR, cDNA, site-specific mutagenesis, transient transfection

三、Background and Specific aims

I. Background and Significance

I) MPS type IVA (Morquio A syndrome) was described simultaneously and independently by Morquio (1929) in Montevideo, Uruguay, and Brailsford (1929) in Birmingham, England. The condition was the entity in which the occurrence of corneal clouding, aortic valve disease, and urinary excretion of keratosulfate were recognized. Tomatsu et al. (1991) cloned and sequenced a full-length cDNA of human placental N-acetylgalactosamine-6-sulfate sulfatase (GALNS). The coding sequence had 1,566 nucleotides which encoded a polypeptide of 522 amino acid residues. The deduced amino acid sequence was composed of a 26-amino acid N-terminal signal peptide and a mature polypeptide of 496 amino acid residues including 2 potential asparagine-linked glycosylation sites.

II) MPS type IVB (Morquio B syndrome) patients present with mild dysostosis multiplex, odontoid hypoplasia, short stature, cloudy corneas, and keratansulfaturia, but no detectable central nervous system abnormalities (Arbisser et al. 1977). Beta-galactosidase (GLB1) activity was deficient in cultured fibroblasts, but galactosamine-6-sulfate sulfatase activity (deficient in classic MPS IV) was normal.

III) GM1-gangliosidosis, also caused by the deficiency of beta-galactosidase, is the alleleic form of MPS type IVB. Clinical features are: (1) severe cerebral degeneration leading to death within the first 2 years of life; (2) accumulation of ganglioside in neurons, and in hepatic, splenic and other histiocytes, and in renal glomerular epithelium; and (3) the presence of skeletal deformities resembling Hurler disease (O'Brien et al. 1965). Oshima et al. (1988) showed that human placental beta-galactosidase had a

coding sequence of 2,031 nucleotides which encode a protein of 677 amino acids, including a putative signal sequence of 23 amino acids and 7 potential asparagine-linked glycosylation sites.

II. Specific aims

Our broad long term objective is to develop therapeutic drugs for most genetic metabolic disorders. In the mean time, we would like to elucidate the mutation causes of those rare genetic diseases. While MPS type IVA lacks N-acetylgalactosamine-6-sulfate sulfatase (GALNS), MPS type IVB lacks beta-galactosidase (GLB1). The allelic form of MPS type IVB is GM1 gangliosidosis, which was also caused by the deficiency of beta-galactosidase. MPS type VI was caused by the deficiency of arylsulfatase B. All the responsible genes had been cloned and well studied, which led us to propose the following specific aims:

I) Mutation analysis of MPS type IVA
II) Mutation analysis of MPS type IVB
III) Mutation analysis of GM1 gangliosidosis

四、Results and Discussion

Mutation analysis of MPS type IVA

We have four patients who were diagnosed to be MPS type IVA. All mutant alleles of those four patients were identified. Of those identified mutations, Met318Arg was most prevalent and accounted for five out of f eight alleles analyzed. The other two mutant alleles are novel and both were deletion mutations. One is a six-nucleotide deletion, resulting in in-frame deletion of two amino acids in exon 1 of GALNS gene, while the other is a complex deletion involving intron 2 and exon 3 region.

Mutation analysis of GM1 gangliosidosis

We have two patients who were ruled out as GM1 gangliosidosis. All mutant alleles, which included three missense mutations and one in-frame deletion, were identified. All four mutations were novel.

五、Self-evaluation

During the past grant period (one year span), we identified all mutant alleles in four MPS IVA and two GM1 gangliosidosis patients. Most identified mutations were novel. We think the mutation data we acquired through this grant support will be helpful for future mutation study and genetic consultation of those patients' family. We think the data acquired is worth publication and we have submitted one manuscript regarding one novel GALNS mutation to the journal of "Human Mutation".

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