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一、中文摘要

Machado-Joseph Disease 簡稱 MJD，是體染色體顯性的遺傳性疾病，屬於漸進性神經退化性疾病的一種亞型。MJD 主要的臨床表徵則為運動障礙、肌肉萎縮、及錐體系路徑症狀等。調控此症狀的基因座落於第十四對染色體的長臂上(14q32.1)，稱為 MJD 基因。在此基因的 3'端轉譯區(3'-translated region)內有一段異常的 CAG 核酸重複序列發生倍增突變(amplification mutation)是造成疾病的原因。近年來國外有多篇關於 MJD 病例的報告，我們近年來從臨床醫師的病歷診斷幫助，再配合實驗室分子生物診斷 MJD 的技術已臻成熟，目前已在國內找到了患有 MJD 疾病的十六個家族。他們的 CAG repeats 範圍從 72-85 個 repeats，我們同時分析了 150 個正常人的染色體，正常範圍是 13-44 個 repeats。本研究的主要成果，一、建立了十株 MJD 病人。未發病人及正常人的淋巴母細胞株。二、建立了在大腸桿菌中表達 MJD 的 His-Tag MJD 載體。並純化 MJD 蛋白。三、我們利用純化 MJD 蛋白製作了多株抗體，我們的實驗證實 MJD 蛋白表達的程度在 MJD 病人。未發病人及正常人的淋巴母細胞株中並無差異。另一方面。我們使用 lamotrigine 的藥物處理 MJD 病人淋巴母細胞發現可部分抑制突變 MJD 蛋白的表達，目前更進一步的實驗正在神經細胞中進行中。

關鍵詞：Machado-Joseph Disease, 神經退化性疾病, 淋巴母細胞株, 多株抗體

Abstract

Machado-Joseph disease (MJD) is an autosomal dominant spinocerebellar degeneration characterized by cerebellar ataxia and pyramidal signs associated in

varying degrees with a dystonic-rigid extrapyramidal syndrome or peripheral amyotrophy as major neurologic signs. Unstable CAG trinucleotide repeats expansion in MJD gene on the long arm of chromosome 14 has been identified as the pathologic mutation for MJD. We have identified 16 MJD affected families. In addition, we have analyzed the range of CAG repeats in 150 control individuals and the CAG repeat number is ranging from 13 to 44 in the control individuals and 72-85 in the expanded individuals. In this proposal, ten lymphoblastoid cell lines (LCL) from patient of different age at onset, at-risk individuals and normal controls were established. In addition, his-tag MJD recombinated clone was established and E.coli expression MJD protein was purified. The polyclonal antisera against MJD protein were raised from rabbits. We have confirmed by the western analysis that the MJD protein expression levels of at-risk members, patients and normal individuals are indistinguishable. In addition, our preliminary results indicated specific inhibition of the MJD protein expression due to lamotrigine drug treatment in cultured cells. In the coming project period, we continue to study the mechanisms involving the drug effect on MJD protein expression inneuronal cells.

Keywords: Machado-Joseph disease, neurodegenerative disorder, lymphoblastoid cell line, polyclonal antibody

Introduction

Machado-Joseph disease is an autosomal dominant spinocerebellar degeneration characterized by a wide range of clinical manifestations, including ataxia, progressive external opthalmoplegia, pyramidal and extra pyramidal signs, dystonia with rigidity, and distal muscular atrophies. The disease manifestations usually start during adulthood, with a mean age at onset of 37.4 year (SD14.1), but the distribution of age at onset is very wide, ranging from 5 to 73 years (Sequeiros and Coutinho, 1993). The disease locus was mapped to chromosome 14q32.1 in Japanese families (Takiyama et al., 1993). However, the pathologic reason of the late onset still remaines to be answered.

Recently, the gene has been identified and shown to contain a CAG repeat motif in the 5'region of the coding sequence, which is selectively expanded in MJD patients. Therefore, MJD is one of the at least ten diseases results from CAG repeat expansions in coding sequences which are translated into glutamine tracts. These diseases include Huntington's disease (HD) (The Huntington's Disease Collaborative Research Group, 1993;Andrew et al., 1993, 1994), spinocerebellar ataxia type I (SCA 1) (Orr et al., 1993; Chong et al., 1994; Chung et al.,

1993), spinal and muscular atrophy (SBMA or Kennedy disease) (La Spada et al., 1991), spinocerebellar ataxia type II (SCA 2) (Pulst et al., 1996; Imbert et al., 1996; Sanpei et al., 1996), Machado-Joseph disease (MJD)/ SCA3 (Twist et al., 1995; Maciel et al., 1995; Kawaguchi et al., 1994), and dentatorubral-pallindoluysian atrophy (DRPLA)(Aoki , et al., 1994; Burke et al., 1994). As yet there is little understanding of how the polyglutamines function either normally or when expanded. However, it was demonstrated that the expanded allele containing the CAG expanded repeats was translated into polyglutamines in the brain with MJD (Trottier et al., 1995). In a transgenic mice study, it was reported that the expanded polyglutamine in the MJD protein inducing cell death and the expanded polyglutamine appeared precipitated in the cell (Ikeda et al., 1996). It was also observed that the cell death induced by the expanded polyglutamine is gene dose-dependent (Ikeda et al., 1996), which is consistent with the clinical manifestations in MJD (Kawakami et al., 1995; Takiyama et al., 1995). However, to our knowledge, no detailed studies on the expressions of the MJD product from Machado-Joseph disease affected and at-risk individuals.

Although MJD was originally described in a Portuguese-Azorean family, it has been reported in a wide distribution in different origins. So far, we have identified 16 MJD affected families in the ataxia families referred to us. In addition, we have analyzed the range of CAG repeats in 150 control individuals. In the preceding experiments, we have observed that the CAG repeat number is ranging from 13 to 44 in the control individuals and 72-85 in the expanded individuals. It is reported that there is a strong inverse correlation between the expanded repeat size and age at onset of the Machado-Joseph disease. Our previous results indicated that the CAG repeat number is related to the age of onset and severity of the disease.

In addition, the trinucleotide repeat mutation in most of the diseases is associated with the phenomenon of anticipation (Duyao M et al., 1995; Jodice et al., 1995), where the disease tends to present at an earlier age in successive generations. Yet, the correlation of repeat length with clinical anticipation is not perfect in the case of MJD gene. Therefore, the detail molecular analysis is valuable in order to understand the pathology of MJD.

Results and Discussion

To investigate the molecular events responsible for Machado-Joseph disease (MJD), in the grant period, we have

accomplished the followings :

(1) Ten LCLs from patients, at-risk individuals and normal controls were established.

(2) Construction of 6xHis-MJD fusion protein expression plasmid was finished.

(3) The QIAexpression purification system from Qiagene was used to overexpress and then purify the fusion proteins from bacteria.

(4) The polyclonal antibodies against MJD full-length protein were raised and used to detect the MJD protein levels from different sources.

Our results of the MJD protein expression levels showed no detectable differences among different lymphoblastoid cell lines. However, the preliminary results from lamotrigine study showed that the expression of MJD protein is decreased upon the drug treatment. However, more controls are needed before reach a conclusion.

Therefore, in the coming project period, we continue the investigation of the expression levels of the MJD proteins under either drug treatment and also under environmental stress. Through the collaboration with Dr. Chin-San Liu, a neurologist in Kuang Tien General Hospital, we will analyze the effect of some drugs, including lamotrigine, which showed clinical benefits, on culture cells. We also plan to introduce some environmental stress, for example oxidation stress and UV damage, to the culture cells. We hope to correlate the expression patterns of the MJD proteins (upon different treatment) to the disease' late-onset. At this time, our experiment is based on the lymphoblastoid cell lines (LCL)and neuron tumor cell line (SK-N-SH), neuroblastoma cells. The molecular analysis of the drug effect on MJD expression will be certainly benefits the MJD patients. We believe that our continuing efforts will be valuable to understand the pathogenesis of the Machado-Joseph Disease.

計畫成果自評

主持人認為研究成果內容已達成相當的預期目標並可作為後續研究之用。藥物處理這一部分的研究結果若可經神經細胞系統實驗的證實。此研究成果將具相當學術價值適合在學術期刊發表。目前實驗及論文正積極進行中。

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