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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。近年來我們已在國內找到了患有**MJD**疾病的二十四個家族。從先前研究計畫中我們已建立** MJD **不同發病年齡患者、未發病者及正常控制組的** Lymphoblastoid cell lines **。我們同時也建立表達** MJD **全長蛋白的載體，殖入大腸桿菌中做大量表達後，製造抗** MJD **全長蛋白的** polyclonal antisera **以進行** MJD **蛋白表達分析。利用這些細胞株，研究不同** Lymphoblastoid **細胞株內，其** MJD **蛋白表達，我們先前實驗顯示異常擴增** MJD **蛋白的表達受** lamotrigine **藥物影響會有** 30%**的降低。因此以我們欲進一步深入研究** MJD **蛋白表達的相關機制，在此計畫中，我們在臨床上有效的藥物對於**MJD**蛋白在神經腫瘤細胞株中的影響，我們長期表達擴增的** MJD **蛋白的人類神經細胞株已建立完成，使用人類神經細胞進行** lamotrigine **藥物處理，實驗顯示異常擴增** MJD **蛋白的表達受** lamotrigine **藥物影響會有至多** 25 %**的降低；環境壓力的改變利用氧化劑** t-Butyl hydroperoxide **處理病人淋巴母細胞株顯示** MJD **淋巴母細胞株比正常淋巴母細胞株有更強的抗氧化耐受性，另一方面已完成建築表達擴增** MJD **與** Histidine **的融合蛋白，將於未來延續計畫時探討**MJD**蛋白是否和其他蛋白有交互作用**.

關鍵詞：Machado-Joseph Disease，**神經退化性疾病，淋巴母細胞株，人類神經細胞株，氧化壓力**

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 24 MJD affected families. During the project period, lymphoblastoid cell lines (LCL) from patients of different age at onset, at-risk individuals and normal controls were treated with potential therapetic drug, lamotrigine. In this grant period, human neuroblastoma cell line expressing the expanded MJD protein has been established. Our results indicated at most 25% inhibition of the expanded MJD protein expression under the lamotrigine drug treatment in the stable neuroblastoma cell line. The treatment of t-Butyl hydroperoxide into lymphoblastoid cells showed that cells expressing expanded MJD is more tolerant to the oxidative stress when compared to normal lymphoblastoid cells. In addition, His fusion expanded MJD clone has been constructed in my laboratory. It will provide excellent material for the following study of looking for the putative proteins(s) that may interact with MJD protein.

Keywords: Machado-Joseph disease, neurodegenerative disorder, lymphoblastoid cell line, human neuroblastoma cell line, oxidative stress

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). 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.

So far, we have identified 24 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.

The treatment of MJD has been so far purely symptomatic. Previous reports suggested that Sulphamethoxazale and trimethoprim (Bactrim) treatment of MJD may have beneficial effect on gait and coordination (Correia et al., 1995). But no biochemical explanation of the drug effects was reported. Lamotrigine, [3,5-diamino-6-(2,3 dichlorophenyl)-1,2,4-triazine] is a phenyltriazine compound, a new antiepileptic drug, which has been widely used as an adjunctive agent in the treatment of complex and simple partial seizures. From the clinical observation, lamotrigine was found to be specifically effective on improvement of some major symptoms of MJD patients. It is of great interest for us to investigate the molecular event involving the drug fuction.

In the previous grant period, we have accomplished the followings :

(1) Six 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.

Results and Discussion

In this proposal, we continued the investigation of the expression levels of the MJD proteins under either drug Lamotrigine treatment and also under oxidative stress. Our experiments were performed on the lymphoblastoid cell lines (LCL) and neuron tumor cell line, neuroblastoma (SK-H-SH) cells. It was reported that neurons are the major affected sites of MJD, Nuclear Inclusions (NI) were seen only in neurons and never in glial cells (Pauson et al., 1997). Therefore, neuroblastoma SK-N-SH cells stably expressing expanded MJD proteins were constructed in our laboratory. We have successfully established a stable human neuroblastoma cell line (SK-N-SH-MJD78), which expresses expanded MJD protein constitutively. Our results indicated at most 25% inhibition of the expanded MJD protein expression under 200 uM lamotrigine drug treatment. We also conducted this experiment in COS-7 cells. Same preliminary results were obtained. Currently, we are verifying the drug effect in a time course experiment. On the other hand, the treatment of t-Butyl hydroperoxide into lymphoblastoid cells showed that cells expressing expanded MJD is more tolerant to the oxidative stress when compared to normal lymphoblastoid cells. In addition, Histidine –fusion full-length expanded MJD clone and truncated clone were constructed in my laboratory. It will provide excellent material for the following study of looking for the putative proteins(s) that may interact with MJD protein.

In the coming project period, we will continue the investigation of the expression levels of the MJD proteins under either drug treatment and also under environmental stress. We also plan to introduce more environmental stress, for example different 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。由於長期表達擴增的MJD**蛋白的人類神經細胞株已建立完成藥物處理這一部分的研究結果已可經神經細胞系統實驗的證實。另一方面建築表達**MJD**與**Histidine**的融合蛋白已完成，將探討是否和其他蛋白有交互作用;氧化劑**t-Butyl hydroperoxide**處理病人淋巴母細胞株顯示**MJD**淋巴母細胞株比正常淋巴母細胞株有更強的抗氧化耐受性,我們將繼續進行相關探討。延續的研究計畫正按照進度展開，且有些具體的初步結果，將對**MJD**此遺傳性漸進神經退化性疾病致病原因及晚發性等病理現象，有進一步的了解。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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