# 行政院國家科學委員會專題研究計畫成果報告

## 轉錄因子 NF-κB 與 AP-1 於神經肌肉疾病中所扮演的角色
## Transcription Factor NF-κB and AP-1 in Neuromuscular Diseases

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### 一、中文摘要

我們使用光學顯微鏡免疫組織化學的方法來觀察轉錄因子 NF-κB 及 AP-1 在正常肌肉及各種神經肌肉疾病的分佈情形。所使用的抗體包括 NF-κB 次單位 p50、p65 的抗體與 AP-1 次單位 c-Jun 及 c-Fos 的抗體。大部分的壞死細胞具有廣泛且強烈的 p50 免疫活性。只有一部分的壞死細胞具有 p65 的免疫活性。這些免疫活性並非存在於巨噬細胞內。在 20-30%的再生細胞中可觀察到 p65 及 p50 的免疫活性。AP-1 中的 c-jun 免疫活性在一部分的再生細胞中有增加的現象，不過在壞死細胞中並不增加。C-fos 的免疫活性並沒有觀察到有明顯的變化。在神經源性疾病不管 AP-1 或 NF-κB 均無明顯變化。NF-κB 及 AP-1 在肌肉的壞死及再生可能扮演著一重要的角色。不同的次單元也具有不同的地位。

關鍵詞：轉錄因子，NF-κB，AP-1，免疫組織化學，神經肌肉疾病

### Abstract

Immunohistochemical study of subunits of transcription factor NF-κB and AP-1 in various neuromuscular disease showed:(1) A majority of necrotic fibers had diffuse immunoactivities(IR) of p50; IR of p65 was only present in a subset. Most of the p50 IR did not co-localize with macrophages; coexistence of p65 and macrophage IR was seen occasionally.(2)In 20-30% of regenerating fibers, there were diffuse p50 and p65 IR. (3) No increase of NF-κB or AP-1 IR was observed in neurogenic disorders. (4) For AP-1 IR, c-jun was increased in a subset of regenerating fibers but not in necrotic fibers. IR of c-fos was not detected in all biopsies. Our results demonstrate that NF-κB and c-jun can be increased in human muscle disease. Different distribution of NF-κB and AP-1 subunits points to their selective role in muscle pathology.

**Keywords**: transcription factor , NF-κB , AP-1, immunocytochemistry, neuromuscular disease

### 二、緣由與目的

NF-κB 及 AP-1 為兩種極重要的轉錄因子[1-4]，它們負責引發及調節許多基因的功能，這些基因包含了與發炎及免疫有關的細胞素、干擾素、MHC 蛋白、生長刺激素、細胞黏著蛋白等。這兩種轉錄因子已被証實在許多生理及病理狀態中扮演著重要的角色。神經肌肉疾病(Neuromuscular Disorders)的成因繁多，其中牽涉到肌肉細胞的退化、死亡、凋亡、再生等過程，在這些過程中發炎、氧化壓力(oxidative stress)均可能扮演著重要的角色[5-6]。NF-κB 及 AP-1 是否參與其中的調節目前還不十分明瞭。本計劃目的在於研究 NF-κB 與 AP-1 在各種不同的神經肌肉疾病中所扮演的角色，我們使用光學顯微鏡免疫組織化學的方法來觀察不同轉錄因子在正常肌肉及各種疾病的分佈情形。所使用的抗體包括 NF-κB 次單位 p50、p65 的抗體與 AP-1 次單位 c-Jun 及 c-Fos 的抗體。所使用的肌肉切片包括正常肌肉，發炎性肌肉病變(inflammatory myopathies)，肌肉失養症(muscular dystrophies)，粒線體肌肉病變

(mitochondrial myopathy)；脊髓側索硬化肌萎縮症(amyotrophic lateral sclerosis)；脊髓肌肉萎縮症(spinal muscular atrophy)等，以包含各大類不同病因的神經肌肉疾病。我們特別注意肌肉神經交接處，壞死細胞，再生細胞，發炎細胞，襤褸紅細胞及空洞細胞處的變化。另外用雙重螢光染色法來觀察與desmin、巨噬細胞的關係。

## 三、結果與討論

Results:

In necrotic fibers of myopathies (identified by lack of desmin IR), there were very strong and diffuse p50 IR. Around 95% of desmin negative fibers had strong diffuse p50 IR. Diffuse p65 IR were also present in around 30% of necrotic fibers. Double labeling with macrophage markers (Ber-Mac 3) showed that most of the p50 and p65 IR in the necrotic fibers did not contain Ber Mac 3 IR, co-existence of the p65 and Ber-Mac 3 IR was seen occasionally.

In 20-30% of regenerating fibers (identified by double labeling with desmin), there were diffusely increased p50 and p65 IR.

There was no increased IR of either antibody in muscles of ALS patients.

In the normal muscle, p65 IR was present at the postsynaptic dormain of neuromuscular junctions(NMJ), where they co-localized with bound $\alpha$ -bungarotoxin. No p65 active form or p50 IR was observed at the NMJ.

C-jun was increased in a subset of regenerating fibers but not in necrotic fibers. IR of c-fos was not detected in all biopsies.

No AP-1 or NF-$\kappa$B IR could be detected in the nuclei in all nuclei.

Discussion:

*NF-$\kappa$B in necrotic fibers:*

One striking finding in our study was the strong, diffuse IR of p50 and p65 in necrotic muscle fibers (identified by lack of desmin staining). Most of the p50 and p65 IR in the necrotic fibers did not contain macrophage IR, indicating these IR were from necrotic muscle fibers rather than from infiltrating macrophages. The same findings were seen in necrotic fibers of all myopathies, suggesting NF-$\kappa$B has a common role in the necrotic process of different human skeletal muscle diseases. In these fibers, some stimuli may augment NF-$\kappa$B activity at both transcriptional and post-transcriptional levels. Cytokines or oxidative stress may be such activating conditions. Sen et al. reported NF-$\kappa$B activation was observed in skeletal muscle derived L6 cells in response to direct $H_2O_2$ treatment (16), suggesting oxidative stress induced NF-$\kappa$B activation can occur in skeletal muscle cell. Blocking the pathway of NF-$\kappa$B activation may be a future therapeutic avenue.

*NF-$\kappa$B in regenerating fibers:*

In 20-30% of the fibers with increased desmin IR, there were also increased p50 or p65 IR. The role of NF-$\kappa$B in the process of muscle regeneration is still unknown. In these fibers with increased desmin activities, previous tissue damage may have occurred and hence the name degenerating-regenerating fibers. Increased NF-$\kappa$B may be the results of previous tissue insults( like being influenced by cytokines), or have an active role in the regeneration, such as increasing the production of growth factors. In other tissues, NF-$\kappa$B may also involved in regeneration (17).

*NF-$\kappa$B in normal neuromuscular junctions:*

Postsynaptically at all neuromuscular junctions there was strong p65 , but no p50 immunoreactivity. To the best of our knowledge, this appeared to be the first time of such demonstration in human muscle. These findings suggest that NF-$\kappa$B is a constituent of NMJ and may function in synaptic signaling. Suzuki et al. recently reported NF-$\kappa$B p50 immunoreactivities were localized in the postsynaptic densities in

rat brain (18), suggesting that NF-κB may have a role in the regulation of signal transmission in the central nervous system (CNS). These findings implies that NF-κB may have a role of synaptic function in both central nervous system and neuromuscular junctions. Different patterns of accumulation between p50 and p65 may indicate their unique physiological role in different synapses.

Lack of detection of NF-κB in nuclei could be due to (a) very small portion of cellular NF-κB translocate into nucleus(9), (b) the access of antibody blocked due to the DNA binding of NF-κB.(c) self regulation, making translocation limited.

The difference between c-jun and c-fos IR is not known. It may indicate their different role in muscle regeneration, or the accessibility of antibodies used.

## 四、計畫成果自評

在免疫組織化學方面大致達成預期的目標，不過在某些肌肉疾病方面則檢體不足，例如包涵体肌炎，其結果無法呈現。在粒線体肌病方面我們在所取得的切片中並沒有觀察到 ragged red fibers 中這些轉錄因子的變化，我們也希望能有更多的檢体後能再次評估。這次結果可說對轉錄因子在神經肌肉疾病所扮演的角色做了一個起始的嘗試，我們將在學術期刊發表，並期望能延續一系列的研究。

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