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• 英文關鍵字	Methylprednisolone; Glucocorticoid receptor; Growth-associated protein 43; JAK; NogoA; Chondroitin sulfate proteoglycans; Neurocan; Spinal cord injury; NogoA
• 中文摘要	合成之糖皮質固醇類藥物(synthetic glucocorticoids)，如甲基去氫可體醇(methylprednisolone,簡稱 MP) 在過去國際急性脊髓損傷第二次臨床研究中(Second National Acute Spinal Cord InjuryStudy)，已被證實可促進急性脊髓損傷的病人之神經修復能力。本計畫旨在了解糖皮質固醇對 GAP-43 基因表現調控之機制。在三年計劃的第一年，我們已建立了 GAP-43 基因促進區(promoterregion)的標的序列，並將 -477~+21 (rGAP43-P1) and -858~-406 (rGAP43-P2) of the rat gap-43 genepromoter 建構於 pGL3 expression vector。在第二年的研究中，我們針對 rGAP43-P2 進行研究，發現其活性與 GAP-43 mRNA 表現吻合，且亦受到 MP 的促進。其他較爲強效的糖皮質固醇, 如 corticosterone 及 dexamethason, 則未能有如 MP 般對 rGAP43-P2 的促進作用。MP 已知具有抗發炎作用，而發炎反應通常與 JAK／STAT 信號路徑有關。我們進而發現，MP 會引發 glucocorticoidreceptor (GR)與 Stat3 在細胞核的交互作用，並且會引發 GR 接合在 GAP43-P2 promoter 上的 Stat3binding site，而 MP 亦會使 GR 與 P53 結合，且會造成 P53 接合到 GAP43-P2 promoter 上的 P53binding site。在 NogoA 基因調控方面，MP 會抑制 AMPA 誘發的 NogoA 基因表現，此一現象亦在 NogoApromoter 活性分析中發現，我們進一步以 promoter analysis 找出了 NogoApromoter 的 MP responsive region，且在脊髓損傷的老鼠中證實了 MP therapy 對受傷脊髓的 NogoA 表現有抑制作用。此外，我們也發現，神經損傷對 NogoA 表現的促進作用及 MP 對於 NogoA 表現的抑制作用在寡突膠細胞(oligodendrocytes) 比在星狀膠細胞(astrocytes)中顯著，顯示 MP 主要爲影響寡突膠細胞的 NogoA 表現。在 inhibitory extracellular matrix 的基因表現研究方面，我們發現 MP 會抑制在 Extracellular Matrix 中，一群會抑制神經生長的 inhibitory extracellular matrix 的基因表現，其中並以對 chondroitin sulfate proteoglycans (CSPG)的影響最爲顯著。我們亦建立了 CSPG gene promoter 的 reporter construct 做爲

	研究其基因表現之高效能篩選平台。因此，本計畫於三年的執行期間，不但發現了糖皮質固醇在脊髓損傷中會促進神經再生的分子機制，更成功的將這些基因的調控機制加以闡明，將可對脊髓損傷以及其它中樞神經損傷的治療策略提供重要的學理依據。
• 英文摘要	Methylpredinisolone (MP), a synthetic glucocorticoid (GC), is the only proven therapeutic agent for acute spinal cord injury (SCI). This project is directed at exploring the genetic mechanisms of MP in the transactivation of growth-promoting genes and transrepression of growth-inhibiting genes. MP promotion of neurite outgrowth was accompanied by upregulation of GAP-43 gene expression in dorsal root ganglion (DRG) neurons under stimulation with kainic acid (KA) in primary culture. The full-length as well as fragments spanning -477~+21 (rGAP43-P1) and -858~-406 (rGAP43-P2) of the rat gap-43 gene promoter have been cloned and subsequently constructed into the pGL3-basic expression vector, followed by transfection into P19 cell-derived neurons. rGAP43-P2–driven 3 reporter expression correlated well with the extent of GAP-43 gene expression upon MP-KA treatment. MP-enhanced GAP-43 phosphorylation was dependent on the Janus kinase (JAK)-mediated signal transducer and activator of transcription 3 (STAT3) pathway via protein kinase C-dependent STAT3 phosphorylation. Furthermore, we found that MP treatment increase interaction of GR and Stat3 in the nucleus, and increase GR and Stat3 binding to the GAP43-P2 promoter. Therefore, these results suggest that MP may enhance GAP43 gene expression via GR-Stat3 interaction. MP effects in promoting neurite growth also involved its effects on the growth-inhibiting factor NogoA and inhibitory extracellular matrix (ECM) proteins. MP reduced the expression of Nogo A, an endogenous inhibitor of neurite growth, in both DRG neurons and astrocytes, and oligodendrocytes. GR-siRNA and application of GR antagonist reversed MP suppression of Nogo A expression. To investigate the transcriptional regulation of MP repression of nogo-A gene expression, we have cloned the rat NogoA promoter fragments rNogoA-P2 (-2183~+7) and rNogoA-P1 (-1115~+7) into the pGL3-basic expression vector. Luciferase activity assay shows that MP treatment decreased AMPA-induced rNogoA-P2-driven reporter expression in both astrocytes and oligodendrocytes. We have identified the MP-responsive region in the nogoA gene promoter. MP therapy in SCI rats also reduced injury-increased NogoA expression. For inhibitory ECM, neurocan, a chondroitin sulfate proteoglycans, which was profoundly upregulated upon excitotoxic insult in astrocytes, was transrepressed by MP in a GR-dependent manner. In summary, we have identified growth-promoting genes and growth-inhibitory genes that are activated or repressed by glucocorticoids respectively during nerve injury, which in turn facilitate nerve regeneration and functional outcome. The genetic mechanisms of glucocorticoid-mediated gene regulation on GAP43, NogoA, and CSPG were elucidated. These mechanisms suggest that glucocorticoid therapy is beneficial in spinal cord injury for nerve regeneration.