脊病變之細胞再生治療的動物模式及分子造影監測

Cell Regenerative Therapy for Spinal Lesion in Animal Model Monitored by Molecular Imaging

中文摘要

脊椎的構造具有包括保護支撐功能的脊椎骨骼、韌帶、關節等，受脊保護的內部構造包括脊髓神經及血管。脊椎骨疾病中，壓迫性骨折常是病患行動困難及疼痛的主要原因，而骨質疏鬆是導致壓迫性骨折最主要因素。另外一個難以治療的脊疾病就是脊髓損傷。雖然每年有許許多多有關脊髓損傷的病理機轉及分子生物學的研究，但是目前仍無法有效的改善病患臨床症狀。因此我們嘗試以 NIH3T3 細胞治療的作爲脊髓損傷治療的另一選擇。NIH3T3 具有局部分化的功能且有自體分泌生長因子如 GDNF 能力。首先我們將 NIH3T3 植入雙重報告基因，再將它用於動物脊椎疾病的細胞治療技術中，再以分子影像學來直接觀察 NIH3T3 的作用軌跡。同時也以分子生物學技術來分析此種治療的可能機轉。本論文包含兩主題。第一主題是利用由胚胎纖維母細胞 NIH3T3，進行脊髓挫傷後之細胞治療。NIH3T3 同時具有能分泌內生性膠質細胞延伸生長因子(GDNF)。第二主題是將去卵巢的 SAMP8 早老化老鼠骨頭，注入利用富含血小板血漿處置後的胚胎纖維母細胞 NIH3T3，引導骨頭新生作用以分子影像來追蹤觀察 NIH3T3 遷移的現象，同時以分子生物技術分析體外及體內 NIH3T3 成骨細胞分化能力及骨化現象。以下兩主題共同特性包括臨床上皆爲脊疾病相關的現代疾病，而且使用相同細胞 NIH3T3 作爲細胞治療的細胞來源摘要。同時以分子影像監測 NIH3T3 遷移能力。並且以分子生物技術分析 NIH3T3 於體外及體內的分化能力。以下兩主題分別敘述。

第一主題利用具有能分泌內生性膠質細胞衍生生長因子(GDNF)由胚胎纖維母細胞 NIH3T3，進行脊髓挫傷後之細胞治療並評估其治療結果。首先在 Long-Eions 大鼠的第十胸椎脊髓以 NYU 撞擊機撞擊，已造成急性脊髓損傷。然後再以帶有雙重報告基因的 NIH3T3 細胞，含有腺嘧啶激酶(Thymidie Kinase)及綠色螢光蛋白(green fluorescent protein)基因，植入第二腰椎脊髓，進而觀察受傷脊髓部分的細胞增生、分化及凋亡預防的情況。另外在活體內以核子醫學或螢光影像追蹤 NIH3T3-TG。在平面以螢光影像追蹤顯影發現，NIH3T3-TG 能在早期術後二小時，即能發現 NIH3T3-TG 從植入處第二腰椎往上遷移 2 公分。而此 NIH3T3-TG 遷移訊號能藉由核子醫學追蹤達 48 小時。同時經由免疫組織化學分析，發現無論於活體外或活體內 NIH3T3-TG 皆能分泌 GDNF。在脊髓損傷三週後，植入的 NIH3T3-TG 不但能從第二腰椎遷移至受傷的第十胸椎，亦能分泌 GDNF，產生抗凋亡作用。最後 NIH3T3-TG 細胞在活體外的細胞分化實驗中，以誘導神經分化的培養液引導，發現其具有與神經細胞型態上及基因上相似的分化潛力。

第二主題是探討骨質疏鬆。骨質疏鬆是現代常見之疾病，骨質疏鬆導致的脊椎壓

迫性骨折，在美國每年約增加 70 萬病例。本研究的目的在於發展一種利用細胞爲基礎的治療方法來引導骨頭再生，並以分子影像及免疫生化反應來追蹤驗證。我們利用含螢光報導基因的胚胎纖維母細胞 NIH3T3-G，先在體外以富含血小板血漿(PRP)引導分化成骨細胞樣細胞，再將此細胞移植入去卵巢早老化的老鼠(OOX-SAMP8)骨內。結果發現此去卵巢早老化的老鼠能免於骨質疏鬆的發生。從分子影像學及免疫生化學表現發現成骨細胞樣分化的 NIH3T3-G 細胞能由植入處遷移至遠端的四肢骨骼。以免疫生化分析反應發現遷移處的骨樣細胞同時表現 osteopontin 及 GFP 在新生骨上，證明 NIH3T3-G 細胞移植在去卵巢早老化的老鼠上能重新增加骨小樑生成及骨密度。有趣的是，PRP/NIH3T3-G 細胞移植能延長去卵巢早老化老鼠壽命，由以上實驗，我們發現一種深具潛力的療法，可能可以治療老年停經後的骨質疏鬆。另外對於因爲先天基因缺陷相對的加速老化的症狀，如 Hutehinson-Gilford Pregeria syndrome，此新療法或許能延長壽命。
綜合以上研究， NIH3T3 細胞不但具有自己分泌 GDNF 的能力，且於體內及體外皆具有分化爲神經樣細胞的能力。同時於體外經由 PRP 引導分化成骨細胞樣細胞。NIH3T3 在植入報導基因後，結合分子影像的組合，是一個具有潛在能力的脊髓傷害及骨質疏鬆細胞治療的研究模式。

英文摘要

The spine anatomy includes the supporting, protecting vertebral skeleton, ligament, joint and the internal protected structures, spinal cord and blood vessel. Osteoporotic spinal compression fracture, one of the many diseases concerning the spine, is often caused severe pain on the patients and therefore induced the difficulties of their actions. Spinal cord injury is another main disease of the spine. Though the topic of the mechanisms of spinal cord injury are studied through pathophysiologist and researched through molecular biology every year, but improvement in the patient''s clinical symptom is still ineffective at present. Therefore, the therapeutic option is that we try to regard NIH3T3 as progenitor cells for cell therapy in spinal lesion. Differentiating activities and endogenously express glial cell line-derived neurotrophic factor (GDNF) are the two characteristics found in NIH3T3. After the transplant of reporter gene into NIH3T3, it is used for cell therapy of animal''s spinal cord injury disease and osteoporosis disease. Then molecule images were used to monitor the NIH3T3 homing activity for cell therapy. So there are two themes included in this thesis. The first is the usage of the NIH3T3 and the assessment of the NIH3T3 cell therapy after spinal cord injury. The second is the utilization of the NIH3T3 as the cell-based bone regeneration approach evaluated through molecular imaging.
The first study is the evaluation of a novel cell-based therapy, the usage of embryonic-derived NIH3T3 cells that endogenously express glial cell line-derived

neurotrophic factor (GDNF) for contusion spinal cord injury (SCI). Proliferation, differentiation and apoptosis prevention were examined following the engraftment of NIH3T3 cells into the spinal cords (L2) of Long-Evans rats subjected to acute SCI at T10 vertebral level by NYU impactor. Dual reporter genes were engineered to be contained by the NIH3T3 cells, namely thymidine kinase (T) and enhanced green fluorescence protein (G), hence NIH3T3-TG. They are used for in vivo cell tracking by both nuclear and fluorescence imaging modalities. It is demonstrated that the transplanted NIH3T3-TG cells at L2 vertebral level have the ability to home two centimeters in distance to the injury site as early as 2 h, and the signals persisted for 48 h post SCI through planar and fluorescence imaging. Immunohistochemical analysis both in vitro and in vivo confirmed that the NIH3T3-TG cells express GDNF. Anti-apoptotic effects were provided by GDNF secreting NIH3T3-TG in the injured cord over the period of 3 weeks. The exhibition of both morphological and genetic resemblance of neuronal cells are found in the NIH3T3-TG cells cultured under the neuronal differentiation medium. As appeared in molecular imaging, GDNF-secreting NIH3T3-TG cells are potential therapeutic models for acute spinal cord injury.
The aim of the second study was the development of a cell-based bone regeneration approach. The study was evaluated by molecular imaging and immunohistochemitry. Methods: Platelet-rich plasma medium (PRP) and intrasosseous transplantation into ovariectomized-senescence-accelerated mice (OVX-SAMP8) were used to pre-differentiated genetically modified NIH3T3 embryonic fibroblasts carrying enhanced green fluorescent protein (NIH3T3-G) were into osteoblast-like cells. Results: The development of osteoporosis was prevented by the PRP-conditioned NIH3T3-G (PRP/NIH3T3-G) engraftment. It is demonstrated through molecular imaging and immunohistochemistry the NIH3T3-G cells migrated from the implantation site throughout the skeleton. It is revealed in the site analysis that the co-expression of osteopontin and GFP are found in the newly formed bone tissue, demonstrating that the bone trabecular architecture and mineral density in treated OVX-SAMP8 mice were repaired. Interestingly, the lifespan was significantly prolonged and showed similarities to congenic senescence resistant strain of mice (SAMR1) in the OVX-SAMP8 mice that received PRP/NIH3T3-G transplantation. Conclusion: The potential of this approach to be applied to treat senile postmenopausal osteoporosis and perhaps inborn genetic syndromes associated with accelerated aging such as Hutchinson-Gilford Progeria Syndrome, and for prolongation of life expectancy in general is very high.