間葉幹細胞的軟骨再生作用

計畫類別：✓個別型計畫    □整合型計畫
計畫編號：NSC90－2314－B－002－353
執行期間： 90 年 8 月 1 日至 91 年 7 月 31 日

計畫主持人：陳敏慧
共同主持人：
計畫參與人員：

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
□國際合作研究計畫國外研究報告書一份

執行單位：台大醫學院 牙醫學系

中華民國 91 年 10 月 20 日
間葉幹細胞的軟骨再生作用

Chondrogenesis of Mesenchymal Stem Cells

計畫編號：NSC 90-2314-B-002-353
執行期限：90年8月1日至91年7月31日
主持人：陳敏慈 台大醫學院 牙醫學系
共同主持人：xxxxxxxx 執行機構及單位名稱
計畫參與人員：xxxxxxxx 執行機構及單位名稱

一、中文摘要

組織工程是目前蓬勃發展的領域，在可預見的未來裡，我們將可看見醫學界藉此用以修復或取代受傷或因老化而被破壞的組織。多年來，學者致力於研究細胞的生長過程，使得組織再生工程有較多的突破與發展的可能，然而這一方面的研究仍停留在初期階段，要使組織再生工程達到成功的境界，有賴學者針對細胞之間的互動加以探討並研發合適的支架（scaffold）材料以及適當的生長激素及細胞的應用。

近年來，學者對於利用取自骨髓的間葉幹細胞以進行組織的修復有著濃厚的興趣，主要是由於此間葉幹細胞廣泛地存在，很容易取得，而且在進行細胞培養時可大量繁殖，間葉幹細胞提供修復骨骼肌肉組織所需的細胞來源，然而許多學者對於關節軟骨的再生及骨折的修復雖然有極大的興趣，目前對於軟骨再生機制的瞭解仍極為有限。

在生物體內複雜的環境中，我們很難確定到底是什麼因素，使得間葉幹細胞可以轉化成為軟骨細胞以及是什麼因素在控制這些細胞終結分化（terminal differentiation）成為多生的軟骨細胞（hypertrophic chondrocytes）。建立生物體外模式以研究這些前體細胞(progenitor cells)的分化機制是很必要的。本研究的目的是提出以體外模式使用間葉幹細胞可以成功地具有軟骨再生作用以建立一個可靠而可重覆得著的細胞培養模式。

本實驗利用人體骨髓幹細胞，將之分層離心後，可取得具有吸附性的細胞，再將此細胞以 trypsin 取下(trypsized)。計算數量而分為三組，依次將之(1)進行單層細胞培養，或(2)進行單層培養14天後再進行細胞集團培養aggregate culture)。利用組織切片及免疫組織化學反應分析軟骨特有的多醣蛋白體及第二型纖維軟骨定性軟骨分化。形成的軟骨以 4% paraformaedehyde 固定以進行掃瞄式電子顯微鏡觀察。可見到軟骨細胞及細胞外基質形成的軟
Abstract

Keywords: mesenchymal stem cells, chondrogenesis

Tissue engineering is an emerging field that allows us to look into the future of medicine, one in which doctors may be able to routinely repair or replace failing or aging body parts. The field is made possible by years of research into processes by which cells grow. Although there is growing excitement in the field of tissue engineering, it is still in its infancy. Success will largely depend on the ability of scientists to figure out complex cellular interactions, then intervening with the right scaffold material and exact growth factors and cells. There is particular interest in mesenchymal stem cells of bone in tissue repair because of the extensive reserve of these cells, their ease of removal, and their expandability in culture. However, despite the interest in regeneration of articular cartilage and repair of fractures, the mechanism of chondrogenesis is poorly understood. The complexity of in vivo conditions hinders identification of the factors that are important in the transformation of mesenchymal stem cells into chondrocytes and those that are important in the control of their terminal differentiation into hypertrophic chondrocytes. Mesenchymal stem cells provide a source of cells for the repair of musculoskeletal tissue. However, in vitro models are needed to study the mechanisms of differentiation of progenitor cells. The purpose of this study is to demonstrate the successful induction of in vitro chondrogenesis of mesenchymal stem cells.

Human bone marrow will be removed and fractioned, and adherent cell culture will be established. The cells will then be trypsinized, counted, and used for two different culture groups as (1) replated in monolayer culture or (2) aggregate culture. Quantitation of chondrogenic differentiation was performed with cartilage-specific proteoglycans stain and Immunohistochemical study with type II collagen. The surface marker of the stem cells were identified with CD105 by flow cytometry. Two types of stem cells were identified. The small one with diameter less than 0.5 μm was not with surface marker CD105. The mature one about 10 μm in diameter was shown with CD 105 surface marker. Slices about 60 μm in
thickness were observed under SEM and the collagen architecture surround the cells were shown with fibrillar interaction similar to real normal cartilage.

結果與討論
多醣蛋白質組織化學反應

本實驗成功地以 TGF-β將骨髓間葉幹細胞導引形成軟骨，並確定有二種大小不同的間葉幹細胞。其中小幹細胞直徑<0.5 μm 無法以 CD105 表面標記示出，而大型間葉幹細胞則可以 CD105 表面顯示標記標示出來。

計画成果自評

本實驗已成功地由取自骨髓的間葉幹細胞導引軟骨之生成。


Haynesworth, S. E.; Goshima, J.; Goldberg, V. M.; and Caplan, A. I.: Characterization of
cells with osteogenic potential from human marrow. Bone, 13: 81-88, 1992. [Medline Link] [Context Link]


