藉由與充質細胞的共培養增強胰腺導管類器官的分化能力

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糖尿病(Diabetes)是種與遺傳、環境因素密切相關的代謝疾病，第一型糖尿病主要是由於自體免疫細胞破壞胰島細胞，導致胰島素合成和分泌不足，造成糖尿病，目前治療的方式為每日施打胰島素，但胰島素價格昂貴、保存及攜帶不易，在臨床上想要根治糖尿病唯一的方法為胰腺移植。但由於供體數量遠遠不足，以及免疫抑制問題，患者須終身服用抗免疫抑制劑；這些治療方法皆有所限制；治療糖尿病還需要有終身免疫抑制(Immunosuppression)的方法。

近年來在糖尿病治療中使用幹細胞有相當大的潛力，幹細胞被視為一種生物材料，其具有恢復組織受損之功能，因此對於糖尿病治療的研究重心著重在如何使用「幹細胞」、「胰臟前軀細胞」來取代捐贈者的胰島細胞，其中Mesenchymal stromal cells (MSCs)具有顯著的免疫調節(immune-modulatory)及促血管生成(pro-angiogenic)之特性，在先前文獻中證明了 MSC 能夠抑制自身免疫疾病及發炎反應中的免疫反應，其中骨髓及脂肪組織來源的 MSC 細胞與胰島的共培養更被證實能增加胰島的分化能力及基因表達，使其成為共培養的理想材料之一。

我的實驗將胰臟導管類器官與不同來源的間充質細胞進行共培養，希望能提高胰臟導管類器官的分化能力，使其分化成具有分泌胰島素潛能的內分泌細胞。目前實驗經由取得小鼠胰臟導管，並且利用 3D 培養環境加入特定生長因子及化合物生成胰臟導管類器官，並且建立能夠有效大量培養類器官的系統；接著使用多種誘導類器官分化之化合物，將類器官分化成能夠分泌出胰島素之內分泌細胞，在免疫螢光染色的結果中顯示，類器官雖成功的誘導出內分泌細胞，但效果並不顯著；因此改用了脂肪來源 MSC 的上清液作為類器官分化的 Condition Medium，並在分化後使用 RT-qPCR 分析分化後的類器官是在胰腺細胞的發育過程中的哪個階段，由 RT-qPCR 的結果顯示利用 ASC 上清液所配置的 Condition Medium 誘導類器官提高 *Krt5* 的基因表達，且有文獻顯示有 *Krt5* 基因表達的導管細胞能分化為 β cells。接下來的實驗我將會使用脂肪、骨髓來源的 MSC 以及 MEF 細胞與類器官進行共培養，以增加類器官分化能力為目標，證明 MSC 是否具有增加胰臟導管類器官的分化能力。

Enhance the differentiation capacity of pancreatic ductal organoids with the co-culture of mesenchymal stromal cells

Date:2023/5/19 Speaker: ZHENG-YI ZHANG Advisor: MING KO CHIANG

Diabetes is a metabolic disorder closely linked to multiple genetic and environmental factors. Type 1 diabetes is mainly an autoimmune disease that destroys pancreatic β cells and gives rise to insufficient insulin synthesis and secretion. Currently, the primary treatment for diabetes is insulin injection; however, this treatment is plagued by its high cost and storage difficulty. Thus, islet transplantation has recently become a popular choice in treating diabetes. However, islet transplantation could only be a viable therapy for diabetes if there is a sufficient supply of insulin-secreting cells. Fortunately, improving the organoids culture technique has provided an exciting platform. This system allows us to understand more about the organs' differentiation process and offers us an opportunity to generate the native cell types that we desire in a dish. Our lab has shown that, with appropriate pharmaceutical agents, the cultured mouse pancreatic ductal organoids can be induced to differentiate into insulin-producing β cells without any genetic manipulation. However, the differentiation efficiency and the health of the differentiated cells still require improvement.

Mesenchymal stromal cells (MSCs) have significant immune-modulatory and pro-angiogenic properties. Previous research demonstrated that MSCs could inhibit immune response and inflammatory response. It has also been shown that when adipose tissue-derived MSCs cells were co-cultured with differentiating iPSC, MSCs can promote the differentiation of stem cells into β cells. In addition, the co-culture of adipose tissue-derived MSCs with islets has been confirmed to increase the differentiation ability and gene expression of islets. Therefore, combining pancreatic organoids and MSCs may be a better treatment for type one diabetic patients. Currently, I have applied the conditioned medium of the adipose tissues-derived MSCs to the pancreatic organoids and examined the expression of several pancreatic genes in the treated cells. I found that the expression of the *Krt5* gene was significantly increased in the organoids treated with the conditioned medium. Interestingly, it has been shown that regenerating β cells are derived from ductal cells whose *Krt5* expression was up-regulated.

In my future work, Adipose-derived MSC, Bone Marrow derived MSC, and MEF will be co-cultured with pancreatic ductal organoids, likely enhancing the

differentiation ability of pancreatic ductal organoids into endocrine cells with insulin-secreting potential. Then I will analyze whether the co-culture will change the pancreatic organoids' gene expression profile and improve the differentiating organoids' survival. The co-cultured organoids and MSCs will ultimately be transplanted to rescue the type 1 diabetic mouse.