

学位論文抄録

Pancreatic differentiation of human iPS cells using a defined and completely xeno-free culture system

(ゼノフリー培養系を用いたヒト iPS 細胞からインスリン産生細胞への分化誘導法の確立)

シャージャラル フセイン
Shahjalal Hussain

熊本大学大学院医学教育部博士課程医学専攻
発生・再生医学研究者育成コース

指導教員

桑 昭苑 教授
熊本大学大学院医学教育部博士課程医学専攻多能性幹細胞学

Abstract of the Thesis

Background and Purpose :

Human induced pluripotent stem (hiPS) cells are considered a potential source for the generation of insulin-producing pancreatic β -cells because of their differentiation capacity. Many of the current differentiation protocols utilize a variety of undefined animal-derived products that may have unknown effects on cell characteristics and differentiation. The potential consequences of transplanting human cells exposed to animal-derived products into patients include an increased risk of graft rejection, immunoreactions, and microbial infections, prions, and yet unidentified zoonoses. Therefore, the establishment of a defined and completely xeno-free culture system with which functional and terminally differentiated endocrine cell types can be generated from hiPS cells is needed for future research and clinical applications.

Methods:

To address these issues, we established for the first time a defined and completely xeno-free culture system to derive INS-expressing β -like cells from hiPS cells using a synthetic scaffold (Synthemax II-SC Substrate, functionalized with short peptide sequences derived from the vitronectin protein, which is covalently linked to the synthetic acrylate polymer, Corning) and serum-free media containing humanized and/or recombinant supplements and growth factors.

Results:

In this study, we have developed a five-step xeno-free culture system to efficiently differentiate hiPS cells into insulin-producing cells in vitro. We found that a high NOGGIN concentration is crucial for specifically inducing the differentiation of cells first into pancreatic and duodenal homeobox-1 (PDX1)-positive pancreatic progenitors and then into neurogenin 3 (NGN3)-expressing pancreatic endocrine progenitors, while suppressing the differentiation into hepatic or intestinal cells. We also found that a combination of 3-isobutyl-1-methylxanthine (IBMX), exendin-4, and nicotinamide was important for the differentiation into insulin single-positive cells that express various pancreatic β -cell markers. Most notably, the differentiated cells contained endogenous C-peptide pools that were released in response to various insulin-secretagogues and high levels of glucose.

Conclusions:

Our results demonstrate the feasibility of generating hiPS-derived pancreatic β -cells under xeno-free conditions and highlight their potential to treat patients with type 1 diabetes.