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学位の種類	博士（　人間生物学　）
学位記番号	博甲第　８７５０　号
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審査組織	グローバル教育院
学位論文題目	Isl1β over-expression with key β cell transcription factors enhances glucose-responsive, hepatic insulin production and secretion (Isl1β と膵 β 細胞に重要な転写因子の過剰発現は、肝臓でのグルコース応答性インスリン産生及び分泌を促進する)

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論文の要旨
Abstract of thesis

Adenoviral gene transfer of key β cell developmental regulators including Pdx1, Neurod1 and Mafa (PDA) has been reported to generate insulin-producing cells in liver.　However, hepatic insulin secretion was transient and glucose unresponsive.

The applicant assumed that the disadvantages of PDA-liver were mainly caused by insufficient reprogramming factor combination. Therefore, the aim of this study was to find additional element(s) required for PDA-liver to extend insulin production and/or to enhance glucose responsiveness.

To this end, the applicant adopted an insulin reporter mouse, MIP-GFP, which expresses green fluorescent protein under mouse insulin 1 promoter. At 7 days of adenoviral PDA transfer to MIP-GFP mouse, liver GFP expressing cells were isolated and purified by FACS through GFP expression with negative control liver and positive control pancreatic islet. Then, she confirmed the successful adenoviral PDA induction in insulin producing liver cells (PDA-cell) by qRT-PCR. She also performed microarray analysis including promoter analysis and pathway analysis to technically verify the microarray data set which also showed the successful exogenous PDA gene activation. Interestingly, qRT-PCR analysis revealed that Ins1/2 gene levels of PDAcellwere 50 fold lower compared to

pancreatic islet. Consistently, microarray gene expression analysis further exhibited that most of β cell hallmark genes were inactivated including endogenous Pdx1. Therefore, this result suggests that PDA-cell would require additional factor(s) to generate functionally enhanced β-like cells.

Next, a screening algorithm was established based on the microarray analysis to select pancreatic enriched genes for PDA-cell. The 5 step microarray-based filtering algorithm yielded 2 final candidate genes: transcriptional factor Isl1 and Elf3. Because splicing variant of Isl1, insulin gene enhancer binding protein splicing variant Isl1β, is reported for the higher insulin transcriptional activity, Isl1β was also added. In vitro insulin promoter analysis showed that Isl1β exerted the highest insulin promoter activity in combination with PDA.

In vivo bioluminescence monitoring was then performed to screen additive effects of Isl1β using MIP-Luc-VU mouse which expresses luciferase under mouse insulin 1 promoter. The results verified that adenoviral PDA+Isl1β transfer produced highly intense luminescence from the liver which peaked at day 7 and persisted for more than 10 days, whereas PDA liver peaked at day 3 and gradually disappeared at day 10.

To check if this effect was reproducible, MIP-GFP mouse was delivered with the same adenovirus combination. Immunohistochemistry analysis confirmed successful ectopic expression of PDA and Isl1β, which correlated with insulin signal but not with glucagon. Isl1β supplementation to PDA increased PDA and insulin transcriptional activity by augmenting both insulin-producing cell numbers in liver and insulin transcriptional activity. Consequently, hepatic insulin production and secretion were increased. Consistent with the previous reports that Pdx1, Mafa and Kir6.2 are target genes of Isl1, transcriptional levels of β cell related genes and functional genes in PDA+Isl1β liver were all upregulated in qRT-PCR analysis. However, liver markers were not further inhibited, suggesting a partial effect.

Finally, the applicant investigated the efficiency of the new combination on STZ induced diabetic mice and PDA+Isl1β treatment ameliorated hyperglycemia for 28 days, and enhanced glucose tolerance and responsiveness. The addition of Isl1β to PDA increased the total duration of insulin transcriptional activity and enhanced hepatic insulin production and glucose responsive insulin secretion. This is thought to be achieved through activating β cell associated genes in liver, including endogenous Pdx1 and Neuord1, which is one of the important indication of conversion. However, uninhibited liver markers and transient effects still suggest this conversion as partial. Nevertheless, it is the first report to achieve glucose responsive insulin secretion in liver under short-term ectopic expression. This entails that one-time intervening treatment could eventually be attainable to generate persistent glucose-responsive insulin production in vivo, as modification of gene combination was efficient. Thus, she proposed that Isl1β is a key additional transcriptional factor for advancing the generation of insulin producing cells in liver in combination with PDA.

She also discussed Isl1 as follows. Isl1 is a well-known insulin transcription factor, involved in pancreatic development, islet cell development, proliferation, maturation, and functional maintenance. Defects in Isl1 are associated with impaired glucose responsiveness, and cause diabetes. Splicing variant Isl1β does not have Lhx-3 binding domain (LBD1, 23 amino acid) near the C-terminus and is exclusively expressed only in β cell lines, whereas canonical Isl1 is expressed in all islet cells. Missing LBD1 domain does not supposedly abolish pancreatic function of Isl1, because Isl1 co-factors in islet majorly cooperate through LIM domain of Isl1, not LBD1 domain. Moreover, LBD1 domain is also described for transcriptional repression by folding Isl1 protein, which could

explain the higher insulin transcription activity of Isl1β. Nevertheless, the same biological role of Isl1 and Isl1β cannot be decided yet, because the function of Isl1β has not been largely elucidated. Therefore, the future study of Isl1β will contribute to understanding of insulin producing cell generation.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant presented a novel findings in her recent original paper and PDD that PDAIβ overexpression induces insulin producing cells in liver with a whole battery of β cell hallmark genes and subsequently enhanced glucose responsive hepatic insulin production. Her new Pdx1, Neurod1, Mafa and Isl1β (PDAIβ) gene transfer is a promising method for future treatment of diabetes. The project and paper were well organized, and expanded her plan in QE2 for further improvement. Discussion with referees included the details about beta cell transcription factors, experimental techniques, and epigenetic plasticity, which is good enough to test her knowledge, ability, and intentions as HBP requirements.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 18 January 2018. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.