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学位の種類	博士（医学）			
学位記番号	博甲第　9636　号			
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学位授与の要件	学位規則第４条第１項該当			
審査研究科	人間総合科学研究科			
学位論文題目	Study on the role of CtBP2 in pancreatic β cell (膵β細胞における CtBP2 の役割及びメカニズムの研究)			
主　査	筑波大学教授	医学博士	高橋　智	
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論文の内容の要旨
Abstract of thesis

In this doctoral dissertation, Ms. Ma describes the role of CtBP2 in pancreatic β-cell. The summary is as follows:

（目的　Purpose）

C-terminal binding proteins (CtBP1 and CtBP2) are transcriptional co-regulators, which are involved in several biological functions including development, cellular survival, and tumorigenesis. Preceding studies have conducted mainly using cultured cells in part because the global deficiency of CtBPs have developmental defects. In addition, none of them unequivocally demonstrated the metabolic roles of CtBPs. In author's supervisor's laboratory, they have found that CtBP2 is a bona fide master regulator of metabolic pathways and that CtBP2 activation robustly ameliorates obesity-induced diabetes as well as hepatic steatosis. Based on this previous observation, the author initiated to explore the biological role of CtBP2 in pancreatic β-cell.

（対象と方法　Materials and Methods）

The author did cell and animal experiments. 293 cells were transfected using lipofectamine 3000 (Invitrogen) and immunoprecipitated by specific antibodies to identify protein-protein interactions. CoIP, ChIP and ChIP-seq were performed using mouse β-cell line MIN6. Insulin gene expression and secretion were evaluated by using MIN6 cells. Pancreas sections of dietary-induced obese mice, genetically obese mice and db/db mice were used to perform immunofluorescence staining. β-cell specific CtBP2 knockout mice were generated to analyze the role of CtBP2 in vivo.

（結果　Results）

The author demonstrated that CtBP2 positively regulated the transcriptional program of insulin gene expression in pancreatic β-cells. Since CtBP2, lacking DNA-binding capability, is postulated to orchestrate transcription through

binding to transcription factors bound on the DNA elements, identification of such transcriptional holocomplexes would be the initial step to reveal the molecular underpinnings of the CtBP2-mediated insulin gene expression. To accomplish this, the author took advantage of a ChIP-seq technique, the unbiased and genome-wide mapping of transcriptional regulators, unraveling that CtBP2 forms transcriptional complex with NEUROD1, a master regulator of insulin gene transcription. The integrative data mining of ChIP-seq combined with an in silico sequence search further unraveled the more complex transcriptional architecture composed of CtBP2, NEUROD1 and epigenomic modifiers that profoundly regulate chromatin remodeling of insulin gene promoter. CtBP2 protein expression levels were markedly decreased in pancreatic β-cells in multiple rodent models of obesity, implicating the decreased transcriptional activity of CtBP2 may cause impaired insulin secretion in obesity. Further supporting this idea, pancreatic β-cell specific deletion of CtBP2 led to β-cell dysfunction accompanied with morphological damage.

（考察　Discussion）

While the amount of CtBP2 seems to be an important role in this study, the activation or inactivation of CtBP2 by metabolic intermediates should provide a more attractive and exciting avenue to better understand the role of this novel system centered by CtBP2. In pancreatic β-cells, glycolytic flux eventually generates ATP as an end-product of the sequential steps of metabolism in mitochondria which is tightly connected to the KATP channel activation and the insulin secretory machinery. Therefore, it is highly plausible that NADH production tightly coupled with this metabolic flux could influence insulin secretion at least in part through CtBP2 activation. In addition, fatty acyl-CoAs or their precursor fatty acids, have been repeatedly reported to influence the insulin secretion with multiple targets of action. Thus, the other aspect of CtBP2 regulation, fatty acyl-CoA mediated inhibition, could also have critical role in the insulin secretion in pancreatic β-cells. The structure-function relationship of CtBP2 can be exploited into the development of novel small molecules for future translational medicine.

The transcriptional complex centered by CtBP2 and NEUROD1 seems to be much larger and more complex than expected. Although the understanding of the full picture of the complex is at early stage of identification, the author extracted some candidate proteins in this study and future work will experimentally clarify the contributions of these proteins. The author's ChIP-seq datasets indicated possible involvement of several transcription factors including PDX1, MAFB, SP1, POU domain family transcription factors and FoxO1 in the CtBP2-driven system. Having observed the atrophic pancreas in long-lived CtBP2 knockout mice, it is intriguing to conceive an idea for future studies to investigate the potential roles of CtBP2 in pancreatic development. Several evidences support this idea, for instance, these transcription factors have been reported to be involved in development, CtBP2 itself has functions to regulate development as exemplified in the phenotype seen in the global knockout mice.

The author's findings support a significant role of CtBP2 in pancreatic β-cells and may be exploited to an exciting therapeutic potential of targeting this mechanism in diabetes.

審査の結果の要旨
Abstract of assessment result

（批評　General Comments）

The applicant identified the critical role of CtBP2 in transcriptional regulation of insulin gene by using cultured cells. In addition, the applicant showed the role of CtBP2 in the pathogenesis of pancreatic β-cell dysfunction in obesity illustrating the possible development of a new attractive therapeutic approach targeting this novel transcriptional system. This analysis adds new information about CtBP2 functions in pancreatic β-cell.

（最終試験の結果　Assessment）

The final examination committee conducted a meeting as a final examination on Feb. 17, 2020. 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）

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.