論文 / 著書情報
Article / Book Information

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Title(English)	Improvement of Primary Human Hepatocyte Cell Attachment through EPAC2 Activation and Unraveling WFS1 Selective Degradation
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種別(和文)	論文要旨
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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	Life Science and Technology 系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of (Engineering)
学生氏名： Student's Name	HELENA, Grace Aprilia	審査員主査： Chief Examiner	Shoen Kume

要旨（和文 2000 字程度）
Thesis Summary（approx.2000 Japanese Characters ）

The dissertation entitled "Improvement of Primary Human Hepatocyte Cell Attachment through EPAC2 Activation and Unraveling WFS1 Selective Degradation" is written in English and contains four chapters. Chapter I, entitled "Introduction," introduces the study in general by including the summaries of two different studies: "Improvement of Primary Human Hepatocyte Cell Attachment" and "Unraveling WFS1 Selective Degradation". The chapter consists of the overview or the big picture of each of the studies with a short explanation of the background of the study, followed by a short explanation of the method and a brief summarization of the results.

Chapter II contains the details of the first study entitled *"Improvement of Primary Human Hepatocyte Cell Attachment"*. The chapter starts with the first section, which provides a background explanation of the importance of primary human hepatocyte (PHH) in pharmacogenetic studies and the problem with partial loss of cell attachment following cryopreservation. The section continues with a detailed explanation of the importance of cell attachment for mammalian culture. Following the general basic introduction, an explanation of the cell attachment signalling pathways, cAMP (Cyclic AMP), is added. A section that explains cAMP downstream activator EPAC is also included. The second section describes the detailed methods used in this study. This section contains the cell culture protocol with details of plating. Details regarding the medium composition and small molecules used were also included. Further details on immunocytochemistry-based data analysis were included together with the high-throughput automation image quantification method. The third section explains the study results that confirm the importance of the following factors in improving PHH attachment: Cell seeding density, cAMP activation, low BSA concentration, and EPAC2 activation. The fourth or the last section of this chapter includes a discussion of the results. This section explains the connection between cell seeding density, cAMP activation, low BSA concentration, and EPAC2 activation on PHH attachment.

Chapter III contains the details of the second study entitled *"Unraveling WFS1 Selective Degradation"*. The chapter starts with the first section, which discusses the literature study and its background. The First part, literature studies, explains the elements included in this study, such as Wolfram Syndrome 1 (WS), WFS1 protein and its relationship with WS, and an explanation of protein degradation in general. Furthermore, it also includes studies about the known mechanism of WFS1 and proximity labeling. The second part explains the research background and why the study needs to be conducted. This part is based on the prior results that WFS1 mutant (W837X and Y652X) showed different degradation times compared

to the wild-type (WT) counterpart in two different cell lines, HEK293T and MIN6. In the MIN6 cell line, WFS1 mutants showed a significantly faster degradation rate than WT. On the other hand, WFS1 mutants and WFS1 WT in HEK293T did not show a significant difference in their degradation rate. This part continues with a hypothesis, and the aim of this study is to elucidate MIN6-specific WFS1 protein degradation. The second section explains the detailed methods used in this study: cell line maintenance (MIN6 and HEK293T), plasmid construction, proximity labeling with TurboID, and PLA (Proximity Ligation Assay). The third section explains the *in-silico* analysis results obtained from the biotinylated LC-MS/MS dataset that identifies the WFS1-interacting proteins found in MIN6 and HEK293T. Further analysis, such as statistical analysis of volcano plot, GO (gene ontology), and KEGG pathway, revealed primary, secondary, and tertiary candidates. Primary candidates were chosen based on their statistical significance and abundance from the derived volcano plot analysis. Secondary candidates were chosen from the comparison of the MIN6 vs HEK293T dataset. Tertiary candidates were chosen from the GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways of MIN6 dataset analysis. These candidates were chosen for further elucidation of MIN6-specific WFS1 protein degradation. PLA result also confirms the direct interaction between WFS1 and primary candidates Sec61a1 and Fkbp2. The fourth section discusses the results and hypothesis on how Sec61a1 and Fkbp2 interact with WFS1.

Chapter IV, entitled "Conclusion," contains the conclusions of both studies and their prospects. From the *"Improvement of Primary Human Hepatocyte Cell Attachment"* study, cAMP synergistic activation by IBMX and Forskolin improved early PHH attachment. Furthermore, the activation of cAMP effector EPAC2 was confirmed to increase PHH attachment potently and might be useful for improving PHH versatility in studies. The "*Unraveling WFS1 Selective Degradation"* study identified primary candidates from TurboID results and confirmed to interact with WFS1: Sec61a1 and Fkbp2. These proteins were upregulated in the pancreas and might be the key to elucidating the MIN6-specific WFS1 degradation mechanism in the future.

In summary, this dissertation elucidated the possible mechanism behind PHH early attachment and possible candidates involved in WFS1 selective degradation. S-220, an EPAC2-specific activator studied in this dissertation, might provide a new means for improving PHH attachment in different culture setups. Furthermore, this dissertation also identified the interacting protein candidates Sec61a1 and Fkbp2 as the possible key players in MIN6-specific WFS1 degradation.