論文の内容の要旨

論文題目　Induction of Pluripotent Stem Cells using Feeder Cells that Secrete Transducible Reprogramming Factors

（細胞導入型初期化因子分泌細胞を用いた多能性幹細胞の誘導）

氏名　　安東赫（あんどんひょく）

Induced pluripotent stem cells (iPSCs) can be generated from differentiated somatic cells by viral-mediated transduction of defined transcription factors, c-MYC, OCT4, SOX2, and KLF4. A number of modified reprogramming devices have been developed. However, all of the methods using viral vectors or plasmid DNAs already contain some probable risks from remained transgenic viral vector sequences, undegraded pDNAs, and potential transgenic insertions. Transduction of exogenously expressed recombinant transcription factors fused with cell-penetrating peptide (CPP) can generate mouse and human iPSCs. In recent years, a number of groups have reported the generation of mouse iPSCs using recombinant proteins purified from *E.coli* and human iPSCs using crude cell extracts. These protein transduction methods do not have a potential risk of insertional mutagenesis due to viral vectors, pDNAs or doxycycline-inducible systems, etc. However, there are some demerits or limitations to the

practical application, because purification of recombinant proteins and establishment of iPSCs colonies with these transduction methods are laborious and time-consuming task compared to those with viral vector-mediated gene transfer.

We therefore explored a practical application of the generation of protein-induced iPSCs using genetically modified SNL feeder cells secreting recombinant proteins (SP-SNLs). For the generation of these transformed SNLs, we constructed mammalian expression vectors pSecTag2 expressing and secreting reprogramming factors. To efficiently transduce these secreted reprogramming factors into target cells, we conceived the idea of adding TAT, protein transduction domain (PTD) or other PTDs will be needed in the expression vectors. However, we also considered the possibility that furin (also known as PACE), a ubiquitously expressed endoprotease in the Golgi apparatus and endoplasmic reticulum (ER), recognizes and cleaves TAT-PTD and some of the reprogramming factors during secretory transition processes. To circumvent this problem, we introduced the mutated sequences to all the furin-recognition sites within TAT-PTD, c-MYC, and SOX2. It has been previously reported that PTD-4, a mutant form of TAT-PTD (we call it TAT4.), shows highly improved efficiency in transducing the bioactive molecules and none of the furin-recognition sequences are found in its peptide sequence. Therefore, we thought that TAT4 peptide can be theoretically applied to our system for the transduction of secreted recombinant proteins with high efficiency. Unlike other reprogramming

factors, OCT4 can be secreted through Golgi apparatus and ER without degradation, because it doesn't contain furin-recognition sequences. However, since c-MYC, SOX2, and KLF4 can be susceptible to proteolytic processing by furin or furin-like proteases, we introduced some mutations into c-MYC and SOX2 to make them furin-resistant: R367K and R424Q for c-MYC, R43Q and R114Q for SOX2. Finally, we utilized a reprogramming factor, KLF5 as a substitute for KLF4 having a furin-recognition site at amino acids R374-R377. Based on the above-described modifications, we finally constructed the expression vectors encoding furin-resistant reprogramming factors and established stable transformant cells, SNLs secreting the transducible four reprogramming factors.

With SP-SNLs, as feeder cells secreting transducible reprogramming factors and recombinant protein producer cells, we succeeded in generating mouse iPSCs from mouse embryonic fibroblasts (MEFs) and human iPSCs from human dermal fibroblasts (HDFs) with high efficiency and without extended generation time compared with viral-transduction. Additionally, we also succeeded in generating human iPSCs from human cord blood $CD34^+$ progenitors. From these iPSCs generations, we confirmed that furin-resistant reprogramming factors could generate iPSCs more efficiently than furin-sensitive ones. These iPSCs generated with SP-SNLs and secreted transducible reprogramming factors had basic characteristics of a pluripotent population.

In conclusion, we have demonstrated that direct delivery of reprogramming proteins by feeder cells can avoid the potential risks of transgenic insertions and will provide greater insight as to the beneficial roles in regenerative medicine.