University of Fribourg / Faculty of Science and Medicine / Department of Chemistry

From urine progenitor cells to induced pluripotent stem cells
A focus on characterization

Mira Stürmlin

Introduction: Rare diseases like differences/variants of sex development (VSD) are difficult to study, therefore personalised cell models can help to better understand these differences. For this purpose, patient cells are reprogrammed into induced pluripotent stem cells (iPSCs). Urine progenitor cells (UPCs) are a possible source with great potential for reprogramming into iPSCs. These cells are easy to obtain, the collection is inexpensive and non-invasive. In addition, UPCs have properties such as clonogenicity, expansion and self-renewal capacity and differentiation potential (1 ,2). UPCs offer many advantages and are a good source of cells that can be reprogrammed into iPSCs.
Methods: Urine progenitor cells from voided urine of three healthy volunteers and two patients with VSD were used for reprogramming into induced pluripotent stem cells. Two viral vector-free methods, namely nucleofection and magnetofection, were used for reprogramming. The UPCs and iPSCs were analysed and characterised quantitatively and qualitatively by means of morphology, antibody staining and quantitative real-time polymerase chain reaction (qPCR).
Results: UPCs could be cultured from female and male participants and one female and one male patient. Three different morphological types of UPCs were seen. Type I was spindle-like cell, type II cobblestone-like and type III had a reticular-like morphology. Using nucleofection and magnetofection, UPCs from the control male individual were successfully reprogrammed into iPSCs. No iPSCs could be generated from the female UPCs. Nucleofection was more efficient than magnetofection, whereas magnetofection resulted in fewer but higher quality iPSC colonies. Colonies with good quality were characterised by sharp colony borders, homogeneity and increased nucleus-to-plasma ratio. The UPCs of the control female individual stained positive for CD73, CD44, OCT4, CD146 and inconsistent NANOG using immunofluorescence (IF). Quantitative characterisation by qPCR revealed that they strongly expressed CD73 and OCT4 and weakly expressed CD146, SSEA-1, NANOG and SOX2. The UPCs of the control male individual stained positive for CD73, CD44, OCT4, and NANOG. Quantitative characterisation by qPCR showed that they strongly expressed CD73, moderately expressed CD146 and OCT4, and weakly expressed SSEA-1, NANOG and SOX2. The iPSCs showed positive staining for alkaline phosphatase, CD73, CD44, OCT4, CD146, TRA-1-81, NANOG and SOX2. Quantitative characterisation by qPCR revealed that they strongly expressed NANOG, OCT4 and SOX2 and moderately expressed CD73, CD146 and SSEA-1.
Discussion: Morphologically different UPC types (type I and type III) appear to differ only slightly in typical UPCs markers. It remains unclear whether the different morphology is due to different origins in the urogenital tract. Female UPCs or type III or the combination of both appear to have a lower potential for pluripotency. Nucleofection was found to be more efficient and faster than magnetofection. Nevertheless, better quality colonies were obtained by magnetofection, possibly due to the gentler procedure.
UPCs can indeed be defined as progenitor cells, as they express certain stem cell markers such as OCT4 and inconsistent NANOG, but not SOX2 and TRA-1-81. They differ from iPSCs in their capacity for self-renewal, differentiation and clonogenicity in that. The iPSCs expressed all stem cell markers examined here and in addition, they expressed some mesenchymal stem cell (MSC) markers, which indicates a certain “memory” of the cells of origin.

Jury:
Prof. Dr. med. Anna Lauber-Biason (thesis supervisor)
Prof. Dr. David Hoogewijs (external co-examiner)
Prof. PhD Serge Nef (external co-examiner)
Prof. Dr. med. Michael Walch (president of the jury)

---

[1] Bento G, Shafigullina AK, Rizvanov AA, Sardão VA, Macedo MP, Oliveira PJ. Urine-Derived Stem Cells: Applications in Regenerative and Predictive Medicine. Cells. 2020 Feb 28;9(3).

[2] Zhang D, Wei G, Li P, Zhou X, Zhang Y. Urine-derived stem cells: A novel and versatile progenitor source for cell-based therapy and regenerative medicine. Genes Dis. 2014 Sep 1;1(1):8-17.