



**Karolinska
Institutet**

Institutionen för Neurovetenskap

Human Pluripotent Stem Cells: Effects of Handling and Microenvironment

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska
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av

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ABSTRACT

Pluripotent stem cells hold great promise in regenerative medicine and drug development due to their developmental potential towards multiple lineages. However, there are still hurdles to overcome before these cells are safe enough for clinical use even though attempts have already been initiated.

To generate pluripotent cell populations for specific clinical purposes, it is crucial to understand the regulatory role of microenvironmental cues as well as factors and signaling pathways that control their cellular phenotype. The main purpose of this work has been to study how pluripotent cells may change due to handling and how microenvironmental cues may influence the characteristics of pluripotent cells.

An effective and robust cryopreservation system is necessary in order to store pluripotent stem cells. We compared a standard freezing method with a novel chemically defined xeno-free solution and found that the number of frozen colonies vs. the number of surviving colonies improved significantly with the new method. Using a live/dead assay we obtained 90-96% viability without any impact on proliferation using the new method, compared with the standard freezing procedure (49%). Furthermore, to enable up-scaling of undifferentiated cells, it is important to passage cells with limited effect on cell quality. We investigated whether different passaging techniques can affect the properties of stem cells. We found that cells passaged in the presence of ROCKi displayed alterations in actin organization. We also found decreased expression of genes encoding common pluripotency markers in cells enzymatically passaged compared to mechanical passaged cells, irrespective of the use of ROCKi, but no correlation to histone modification on the promoters of these genes. We found that the effect of enzymatic passaging and ROCKi were at least in part reversible.

It is relevant to investigate differences between early and late passages when it comes to chromatin state and expression of stem cell markers. We analyzed the gene expression and histone acetylation levels in the promoters of well-defined transcription factors associated with pluripotent state. The acetylation analysis was focused on lysines 8 and 12 on histone H4, since acetylation of these residues has been associated with the interaction of the SWI/SNF complex as well as DNA damage. We could detect variations in histone acetylation levels and gene expression between different cell lines. However we found no significant correlation between either histone acetylation and gene expression levels, nor with these parameters alone and passage number. This suggests that other parameters should be evaluated for screening of clinically suitable pluripotent stem cells.

Finally, prolonged culturing of stem cells *in vitro* may select for cells with genetic changes which often result in growth advantages by acquired mutations, called culture adaptation. By performing a comparative *in vivo* and *in vitro* study on human embryonic stem cell lines we were able to identify chromosomal aberrations that had occurred *in vivo*. We also found an amplification of the whole X chromosome in cells differentiated *in vivo*. The potential of precancerous mutations in *in vivo* conditions is important to consider for safety measures, and underlines the necessity to remove all pluripotent stem cells from differentiated populations that will be transplanted.