Popularized Summary

The adult human body is composed of trillions of cells. Most of these cells are highly specialized, exerting thousands of unique functions in one giant, highly complex collaboration. Remarkably, these dedicated cells all stem from a tiny human embryo, which itself originates from a single fertilized egg cell. The early embryo contains a small cluster of stem cells, that are capable to form all different cells of the adult body. This unique ability is called pluripotency. Stem cells also expand over time, they divide and produce copies of themselves, while still remaining pluripotent, which is known as self-renewal. Due to these valuable properties stem cells have generated much excitement in medical research, both regarding their clinical potential, but also for expanding our understanding of early human development.

Following optimization of the right growth medium and substrate, it has been possible to isolate and maintain embryonic stem cells (ESCs) in a petri dish from both mouse (mESCs) and human (hESCs). While both are derived from early stage embryos, stark differences were observed in their characteristics. Although both were pluripotent, it seemed hESCs were more heterogenous within and between lines, with respect to their pluripotent abilities. Other differences were also observed, such as accessibility of their DNA and opposing reactions to certain compounds. To distinguish the two, the terms naive and primed pluripotency were adopted. In light of possible future clinical applications, it would be more desirable to possess a homogenous and unspecified pluripotent cell source that can be directed to form all tissues of the body reliably. The aim of many research groups has therefore been to study differences between primed and naive pluripotency and to try to obtain hESCs with more mouse-like naive characteristics.

In this context, we investigated several approaches for the direct derivation of naive hESCs from the ICM, as well as the conversion of primed hESCs towards naive pluripotency. Different culture conditions were tested, however none were able to support direct derivation of naive hESCs. Nevertheless, we were able to obtain cells with naive-like properties from primed hESCs in specific conditions. These involved the addition of a protein known as WNT5A, which is known to counteract cell specification mechanisms. Our findings suggest that the outcome of WNT5A addition heavily depends on the developmental time window in which it is applied.

Primed and naive stem cells also differ in their DNA accessibility. Many different molecular mechanisms associate with DNA in the cell nucleus, exerting precise control over gene expression. These processes are crucial for governing when and how much gene product is constructed. One major form of control is performed by histones, specialized protein complexes that help condense and organize DNA. Histones undergo chemical modifications known as histone post translational modifications (hPTMs) that can influence how they interact with one another and also determine how tight or how loose the DNA is wound around them. Histone PTMs can also attract other modifying factors which in turn also influence DNA accessibility. Until now, most studies have focused on investigating only one or few hPTMs at a time. However, in this thesis we applied a more comprehensive approach. Using a technique known as mass spectrometry we were able to analyze multiple histones and their hPTMs from numerous cells simultaneously. Specifically, we applied this method to investigate a wide-range of histone PTM changes that occur during the transition of primed hESCs towards naive pluripotency. We were also able to compare our results to previous results from mESCs, discovering conserved mammalian hPTM signatures for naive pluripotency. Our analysis delivered the first roadmap of hPTM changes during naive conversion, a valuable resource for studying histone dynamics in human stem cells.

Finally, we investigated possible methods to overcome certain less-favorable properties of primed hESC, including culture heterogeneity and specification bias. To this end, we investigated the effects of specific culture conditions, known as DhiFI, both on existing lines and during human stem cell derivation. Interestingly, DhiFI-hESCs were shown to be distinct from primed and naive hESCs, but more closely related to primed counterparts. Yet, compared to primed hESCs, DhiFI hESCs were less specialized and more homogenous in culture. In addition, we evaluated the capacity of DhiFI hESCs to form neuronal and cardiac precursor cells. Overall, DhiFI-hESCs performed similarly to primed hESCs and were even able to generate beating cardiac cells at faster rates. This was in stark contrast to naive hESCs, which performed poorly in both protocols. Overall, we demonstrate that DhiFI conditions counter drawbacks of both primed and naive pluripotency, representing a distinct, less heterogeneous starting point for possible clinical applications of human stem cells in the future.

Taken together, we provide valuable insights into the dynamic molecular landscape within hESCs, whilst exploring novel conditions under which pluripotency can be induced and maintained.