Written Statement for 7/24/2013 SWG Meeting

Dear Dr. Lomax,

Steve Peckman request that I email you to explain why my lab is interested in using the OHSU SCNT hESCs, as this may be important for the decisions of the CIRM Standards Working Group.

My lab is using the inactive X chromosome to study the epigenetic stability of the pluripotent state in the human system.

The somatically silenced X chromosome (the Xi) is consistently reactivated when somatic cells of the mouse are reprogrammed to iPSCs such that female mouse iPSCs carry two active X chromosomes (XaXa) just like female mouse ESCs, and X-inactivation is induced upon differentiation of these cells. However, in 2010, we demonstrated that the Xi is not reactivated during human cell reprogramming. Therefore, female human iPSCs carry an Xi with XIST RNA coating at early passage. We also noted that silencing of XIST occurs in human iPSCs over time in culture, leading to subsequent partial reactivation of the Xi that cannot be reversed upon induction of differentiation. Such an epigenetic instability of the Xi has so far not been described for any somatic cell type, but is also characteristic for human ESCs. Thus these human pluripotent cells appear epigenetically instable for reasons that are still unclear.

However, there is an important distinction between human ESCs (fertilization derived) and human iPSCs regarding the X chromosome. Human ESCs but not human iPSCs, can be in the XaXa state. The prevailing model argues that XaXa human ESCs represent the developmentally most immature state, and undergo X-chromosome silencing upon differentiation, mirroring what we know from mouse ESCs, or upon long term culture. Subsequently, just like XiXa iPSCs, such XiXa hESCs often lose XIST expression resulting in partial erosion of the inactive state (reactivation).

In my lab, we are therefore interested in understanding what happens to the somatically silenced X chromosome when differentiated cells are reprogrammed by SCNT. The key question is: are these SCNT-ESCs more similar to iPSCs or fertilization-derived ESCs with respect to the epigenetic state of the X chromosome. Furthermore, it has been shown in mouse reprogramming that the active X chromosome becomes deregulated during SCNT-based reprogramming, and we would like to address this problem in the human system as well.

We believe that the comparison of the epigenetic states between fertilization-derived ESCs, SCNT-ESCs and human iPSCs is important for a better characterization of these cells and understanding of their epigenetic nature.

Please let me know if you have any more questions.

With regards,
Kathrin Plath
Associate Professor at UCLA