Porcine induced pluripotent stem cells (piPSC) for expanding the use of swine in biomedical research

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Our goal is to create porcine pluripotent stem cells, i.e. ones capable of differentiating into all cell types of the body that can expand the use of swine as a biomedical model for studying human disease. It is well established that mouse embryonic stem cells (ESC) are an excellent source of material for successful cloning and for incorporation into chimeras. However, the establishment of porcine ESC from the embryos has proven to be elusive. There has been a similar lack of success with other ungulate species. Establishing a technology for deriving induced pluripotent stem cells (iPSC) from farm animals will allow the gene knock-in/knock-out methods that have revolutionized mouse genetics to be applied to farm species. Importantly pig is a potentially useful model for studying human pathologies due to similarities in organ size, immunology and whole animal physiology between the two species. If the safety and efficacy of stem cell transplantation is to be tested in an animal model before being applied to humans, the pig would likely be a species of choice. The ability to derive porcine (p) iPSC

lines from a particular outbred animal and conduct tissue transplantation on the same pig later and follow the success of the transplant over the course of months or even years would be a particularly valuable advance. Additionally the ability to provide piPSC from animals with valuable traits would provide a permanent source of cells for clonal propagation that would likely avoid the inefficiencies and problems arising from somatic cell nuclear transfer (SCNT), where the vast majority of cloned offspring die or are developmentally abnormal. We have created piPSC from embryonic fibroblasts and umbilical cord mesenchyme by a similar strategy used for the mouse and human, namely ectopically expressing reprogramming genes in somatic cells. The piPSC resemble human ESC, express the typical gene and surface antigen markers of ESC, proliferate continuously in culture, possess high telomerase activity, form embryoid bodies, and differentiate along the three main germ line lineages. Our aim is to demonstrate that piPSC can be directed to differentiate along defined lineages, specifically towards neuronal tissue, hematopoietic lineages and various mesoderm derivatives including cardiomyocytes by using protocols based on those used successfully with human and murine ESC. These experiments will allow such cells to be used for tissue grafts that are matched genetically to recipients and tested for their safety in transplantation. We shall also establish parameters for routine gene targeting in piPSC, with the ultimate goal of creating genetic models for human diseases where mouse models are inappropriate. In summary, the piPSC lines developed will have enormous utility for exploiting the pig as a model in human pre-clinical applications.

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