

Generation and utility of hepatocyte-like cells from pygmy sperm whale (*Kogia breviceps*) induced pluripotent stem cells.

Emily M. Allen, Annalaura Mancina, Danforth A. Newton, John E. Baatz, Demetri D. Spyropoulos.

Marine Biomedicine and Environmental Science Center, Medical University of South Carolina, Hollings Marine Laboratory, 331 Ft. Johnson road, Charleston, SC, 29412, USA.

The pygmy sperm whale (PSW; *Kogia breviceps*) is the second most commonly stranded cetacean in the Southeastern United States. The PSW is a deep diving mammal (2km) that spends limited time at the surface thus restricting access for biological studies. For example, it is possible that their extreme lifestyle requires a resilient physiology that makes them more susceptible to environmental insult. However, sample availability would be required to test this possibility.

We have focused on efforts to utilize rare samples made available by PSW strandings to study normal cell biology and response to stressors. Unfortunately, these samples produce low numbers of primary cells with limited expansion capacity. To better understand PSW biology and test hypotheses regarding their strandings we have established PSW induced pluripotent stem (iPS) cells that will be differentiated to primary cell types for studies of normal physiology and responsiveness to stressors.

Heavy metal exposure has been argued to be a cause of PSW stranding/lethality. To test this hypothesis, we are developing iPS-derived PSW hepatocytes for toxicity to determine if PSW cells are more sensitive than their human counterparts. In humans, hepatocyte-like cells generated from embryonic stem (ES) cells and from iPS cells have been generated and used for various studies and even considered for transplant therapies. We have generated PSW iPS cells from fibroblasts derived from lungs from stranded/deceased individuals through the activation of 4 stem cell factors. These iPS cells are being cultured to generate hepatocyte-like cells to test the efficacy of current protocols developed for humans.

Successful creation of PSW hepatocyte-like cells will provide us with a reagent to assess heavy metal toxicity in marine mammals, which has potential to aid the prediction, mitigation, and prevention of stranding events.

We are grateful to Wayne McFee and James Powell (NOAA's National Centers for Coastal Ocean Science) for providing samples and Colleen Bryan and Steven Christopher for raising the issue of heavy metal toxicity and strandings. This study was performed under permit 932-1489-10 from the National Marine Fisheries Service (NMFS) and supported by awards from NIST.