Background

Oligodendrocytes
- Myelin-producing cells in CNS.
- Develop from oligodendrocyte precursor cells (OPCs).
- Insulate axons and aid in neuronal communication.
- Limited regenerative capacity in adults.

Demyelinating Diseases
- Include multiple sclerosis and spinal cord injury.
- Myelin loss causes inefficient neurotransmission, resulting in pain, difficulty moving, and tremors.
- Currently no cure – medications may slow disease progression.

OPC Transplantation in Animal Models
- Transplanted OPCs differentiate into myelinating oligodendrocytes in Shiverer mice, and transplantation is associated with increased survival[1,2,3].
- Human ESC-derived OPCs can improve recovery of cervical spinal cord injury in a rat model[4].

Methods

TALENs Olig1/2 Specific Knockout of Porcine Blastocyst

KO pig blastocyst

Sprint GFP-labeled stem cells into blastocyst

TALENs Olig1/2 Specific Knockout of Porcine Blastocyst

Immunofluorescence

Harvest embryos, dissect organs, embed tissues in OCT, cryosection, and immunohistochemistry.

Fluorescent microscopy with Nikon Eclipse Ti-E and Nikon C2 confocal.

Image processing and analysis with Nikon NIS-Elements Advanced Research and FIJI.

Tissue processing, immunohistochemistry, microscopy, and image processing was also performed on day 28 wild-type porcine embryos for comparison to the complemented embryo.

Results

Findings
- GFP+ cells colocalize with Sox2 in the chimera, suggesting a neural stem cell phenotype.
- Neural stem cells can ultimately differentiate into oligodendrocytes, but at this time point it is too early to determine cell fates.
- Olig2 and Nkx2.2 expression in the ventricular zone (VZ) of the wild type is consistent with that of other animal models.
- Olig2 and Nkx2.2 colocalize in the ventrolateral spinal cord of the wild type, suggesting an OPC phenotype.
- Sox2 and RC2 expression throughout the spinal cord VZ suggests a radial glial phenotype.

Future Directions
- Continue interrogating the chimera, using the wild type as a reference.
- Generate additional chimeras for replicability.
- Ensure no incorporation occurs in the germline.
- Ultimately substitute GFP-pig cells for human induced pluripotent stem cells for transplantation into patients with demyelinating diseases.

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References