**Introduction**

Skin-derived progenitors (SKPs) are capable of generating both neural and mesodermal progeny in vitro: neurons, Schwann cells, adipocytes, osteocytes, and chondrocytes, thus exhibiting characteristics similar to embryonic neural crest stem cells. Porcine SKPs show distinct transcriptional profiles when compared to neurospheres/neural stem cells in central nervous system (CNS) and skin-derived fibroblasts, indicating a novel type of multipotent stem cell derived from the dermis of the skin (Zhao & Prather, 2011). However, it remains unclear whether SKP cells can produce ectoderm and mesoderm lineages or other germ layers in vivo, although oocyte-like structures can be induced from porcine SKPs in vitro.

Embryonic chimeras are a well-established tool for investigating cell lineage determination and cell potency through normal embryonic development. The purpose of this study was to test the in vivo developmental potential of porcine SKPs by chimera production.

**Hypotheses**

- Porcine skin-derived progenitors (SKPs) have a broader developmental potential than expected before (neural and mesodermal lineages).
- Pre-compact (4-cell and 8-cell) stage embryos can reprogram or enhance the developmental potential of porcine SKPs through chimera production.

**Materials & Methods**

- Porcine SKP cells and fibroblasts were isolated from the back skin of day 35-50 eGFP male transgenic fetuses (Zhao et al. 2009). Neurospheres (NSCs) were derived from the brain tissue of the same fetuses and cultured in the serum-free medium (DMEM/F12+EGF+bFGF+B27).
- Individual cells or clusters of male GFP transgenic SKPs, NSCs and skin-derived eGFP-expressing fibroblasts were injected into pre-compact (4-Cell and 8-Cell stages) IVF embryos, respectively, and then transferred into corresponding surrogates 24 hours post-injection (Figure 1) (Zhao et al. 2010).
- Additional injected embryos were cultured in PZM3 medium for another two days until the blastocyst stage and subsequently fixed by 4% paraformaldehyde and stained with Hoechst 33342.
- Genomic DNA was extracted from various tissues of putative chimeric piglets and subjected to PCR amplification.
- Tissue samples were fixed by 10% formalin and embedded by paraffin, and then cross-sectioned and immunostained with anti-GFP antibody (Abcam, ab290).

**Results**

- Some of the SKPs injected into embryos migrated throughout the host blastocysts, whereas the SKP-derived fibroblasts and neurospheres stayed as a clump in the embryos two days post-injection (Figure 2).
- After embryo transfers fetuses and piglets were recovered (Table 1). The eGFP transgene was detected in two SKP-derived female piglets but not in NSCs- and fibroblast-derived fetuses and piglets (Figure 3).
- The eGFP transgene showed up in various tissues representing derivatives of three germ layers in SKP-derived female piglets (Figure 4).
- The SRY gene was detected in some of eGFP positive tissues, confirming the chimerism of SKP-derived female piglets (Figure 5).

**Conclusions**

- Porcine SKP cells can migrate and disperse into different locations of host embryos possibly through the process of early embryonic compaction.
- Porcine SKP cells can contribute to various cell types of the three germ layers through chimera production, and have a broader developmental potency than previously expected.
- Neither SKP-derived fibroblasts nor neurospheres can integrate into the host embryos and undergo further development.

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**References**