Isolation of multipotent stem cells from porcine skin

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Background: Stem cells from pigs may provide an efficient means for the production of transgenic swine. Currently nuclear transfer is very inefficient due, in part, to the failure to reprogram the donor cell nucleus from differentiated somatic cells. It has been reported that undifferentiated stem cells may be more easily reprogrammed leading to more efficient transgenic animal production. Therefore, a stem cell population from the porcine species may be an asset for the efficient production of transgenic pigs.

Ojectives: To isolate stem cells from fetal skin, and study their proliferation and differentiation potential *in vitro*.

Results: Using a technique that has been used to isolate floating sphere stem cells from mice, we isolate floating spheres from fetal pig skin. These cells expressed the neural progenitor marker, nestin, as well as genes that are critical for maintaining pluripotency such as Oct4 and Stat3. These skin-originated sphere cells can proliferate for several months *in vitro.* Data from seven isolations revealed a cell doubling range of 55-140 hours. Following two weeks induced neuronal differentiation. a subpopulation of these cells expressed ßIII-tubulin (neuron marker), and were morphologically consistent with neurons (Fig. 1A), another subpopulation of the cells expressed GFAP (astrocyte marker), and were morphologically consistent with astrocytes (Fig. 1B). The expression of these markers was confirmed by a flow cytometry study and real time RT-PCR (data not shown). After culturing, for four weeks, in a medium that was previously reported to induce stem cell differentiation into adipose cells in mice, approximately 2-3% of the differentiated skin cells were positive for Oil Red O staining (Fig 1C), an index of lipid production, they also expressed leptin (Fig. 1D), a hormone produced in adipose cells.

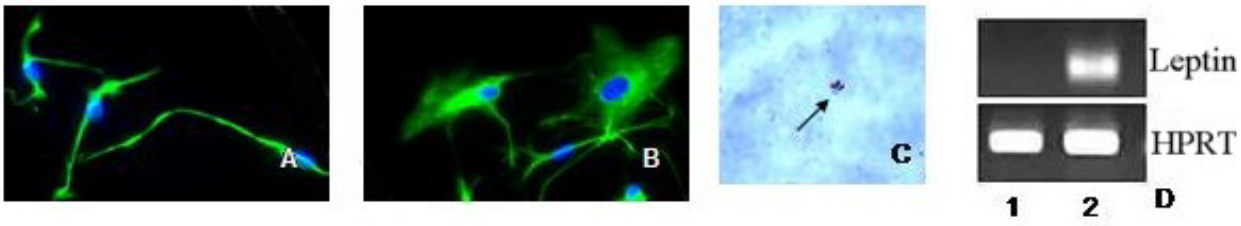

Figure 1: Expression of ßIII-tubulin (A) and GFAP (B) following induced neuronal differentiation. Cells stained positive for Oil Red O (C), and expressing leptin (D) following induced differentiation Lane 1:RT-PCR product from cells before, and lane 2 : after induced differentiation in the adipocyte differentiation medium.

Conclusion: Our study suggests that the isolation of porcine stem cells is feasible. When cultured in specific medium, these cells can differentiate into the neuronal, and mesenchymal lineages *in vitro.*

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