ALTERING DONOR CELL METABOLISM TO BE MORE SIMILAR TO BLASTOMERE METABOLISM INCREASES DEVELOPMENT OF CLONED EMBRYOS TO THE BLASTOCYST STAGE

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Abstract
One of the limitations to development of somatic cell nuclear transfer (SCNT) embryos is thought to be differentiation or metabolic status of the donor cell. Several types of donor cells have been used for SCNT and the most efficient cell type used is individual blastomeres from an embryo. However, blastomeres can be troublesome to culture. Additionally, elaborate genetic modifications, those beyond adding a transgene or knocking out a gene, are difficult to perform on blastomeres. The most common type of cell used for SCNT is fetal-derived fibroblast cells (FC). FC allow for culture over a short period of time and genetic manipulation; however, resulting development is generally low. Our goal was to manipulate the already differentiated FC to behave metabolically as a blastomere. First, we cultured the FC in DMEM with 10% FBS with or without PS48; a small molecule that activates phosphoinositide-dependent protein kinase-1 (PDK1). It has been shown that early embryos have considerable PDK1 activity, i.e. the Warburg effect. Thus by adding PS48 to FC we would hope to activate PDK1 in the cells and thus encouraging them to metabolically mimic blastomeres from early embryos. FC were cultured with 5 µM PS48 for 7 days and the abundance of transcripts for HIF1AN, LDHA, LDHB, NANOG, and PIK3CB was higher than controls; while MTOR was less abundant. While this represents only a single biological replication and a single time point, it shows that PS48 can alter gene expression after only 7 days. FC were cultured for 3-5 days with or without 5 µM PS48 and used as donor cells for SCNT. The SCNT embryos were cultured to the blastocyst stage and development and total cell number was recorded. While there was no difference in development to the blastocyst stage, the cells cultured with PS48 produced blastocysts with more cells 37.5±1.3% vs 32.3±1.4% (SAS 9.3 GLM procedure). Embryo transfer with these PS48 treated donor cells to two surrogates resulted in litters of 8 and 9 piglets. Next we cultured FC in media designed for embryos. FC were cultured in DMEM 10% FBS with 10% FBS with or without PS48 or the traditional FC media (DMEM 10% FBS with 5 µM PS48) for 3-5 days. FC from these treatments were used for SCNT. While there was no difference in the total cell number of the resulting blastocyst stage embryos, FC cultured in the embryo culture medium developed to the blastocyst stage at a higher percentage than the control (28.4±3.7% vs 13.9±5.9%) (SAS 9.3 GENMOD procedure). After treating FC to alter their metabolism to be more similar to blastomere metabolism may enhance the ability of these cells to direct development after SCNT.

Rationale
The most efficient cells type to use for SCNT is a blastomere from an early embryo. However, limitations on culturing this cell type led to using somatic cells, specifically FC, because they could be easily genetically engineered prior to SCNT. Thus with this set of experiments we cultured FC in the presence of PS48, or a combination of PS48 and embryo culture media to try and revert the somatic cell metabolism into becoming more blastomere-like to increase the developmental efficiency and quality of the cloned embryos.

Materials and Methods

Cell Culture and Embryo Culture

Experiment 1
Fibroblast cells were cultured in DMEM with 10% FBS with or without 5 µM PS48 in 5% CO₂, 5% O₂, N₂ balance for 7 days at which time samples were snap frozen. Later RNA was extracted with qiagen Rneasy micro kit(74004) and cDNA synthesized for real-time PCR.

Experiment 2
FC cultured 3-5 days in DMEM with or without 5 µM PS48 were detached from wells using 0.05% trypsin and used for SCNT. Following SCNT cloned embryos were cultured in MU-1 (Porcine embryo culture media) to D6. Blastocyst development was recorded and total cell number was counted using Hoechst 33342.

Experiment 3
FC were cultured in either DMEM 10% FBS with 5 µM PS48 in the same conditions as the previous experiment, or in MU-1 (embryo culture media) with 10% FBS and 5 µM PS48. Both sets of cells were used for SCNT. They were cultured in MU-1 until d6 at which point development to blastocyst and total cell number was assessed.

Results

Experiment 1. Transcript abundance was normalized to YWHAG, and then presented relative to the non-PS48 cultured cells.

<table>
<thead>
<tr>
<th>Gene</th>
<th>PS48 5µM</th>
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<tbody>
<tr>
<td>HIF1AN</td>
<td>2.29±0.22</td>
</tr>
<tr>
<td>LDHA</td>
<td>1.59±0.13</td>
</tr>
<tr>
<td>LDHB</td>
<td>2.35±0.18</td>
</tr>
<tr>
<td>NANOG</td>
<td>1.75±0.1</td>
</tr>
<tr>
<td>PIK3CB</td>
<td>1.63±0.09</td>
</tr>
<tr>
<td>MTOR</td>
<td>0.47±0.07</td>
</tr>
</tbody>
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Experiment 2. Total cell number of SCNT embryos derived from donor cells cultured in PS48 or control DMEM.

![Bar graph showing total cell number of SCNT embryos derived from donor cells cultured in PS48 or control DMEM.](Image)

Experiment 3. Percentage of SCNT embryos that developed to the blastocyst stage DMEM or MU1 containing PS48.

![Bar graph showing percentage of SCNT embryos that developed to the blastocyst stage DMEM or MU1 containing PS48.](Image)

Conclusions

The addition of PS48 to donor cell media does effect metabolism of donor cells and subsequent development of SCNT embryos. Also, culturing the donor cells in media designed for embryo culture with the addition of PS48 leads higher rate of SCNT embryos developing to blastocyst when compared to embryos that had donor cells cultured in traditional FC culture media.

![Image of animal reproductive biology group](Image)