Scriptaid and MG132 Improve Development of Porcine Embryos Reconstructed by Somatic Cell Nuclear Transfer

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Introduction

The efficiency of somatic cell nuclear transfer (SCNT) in pigs and other large animal species is low and is influenced by many factors. Maturation promoting factor (MPF) activity in the recipient oocyte is one of the most important factors for regulating nuclear remodeling and reprogramming of donor nuclei. Metaphase II (MII) oocytes have high MPF activity. High MPF activity in the recipient oocyte has been shown to produce nuclear envelope breakdown and chromosome condensation of somatic cell nuclei. Furthermore, nuclear remodeling patterns, such as formation of the pronucleus, are greatly influenced by MPF activity and are closely related to efficient reprogramming of donor cell nuclei, which permits normal embryo development. When MII oocytes are activated by sperm penetration or artificial stimuli, MPF activity is decreased, which in turn may be detrimental to reprogramming of donor nuclei and subsequent embryo development. MG132 is a proteasome inhibitor and prevents MPF inactivation by blocking cyclin B degradation. It has been shown that MG132 treatment during oocyte manipulation improved the development of SCNT embryos by conserving MPF activity in reconstructed oocytes (You et al. 2010).

In addition, treatment of SCNT pig embryos with scriptaid, a histone deacetylase inhibitor, greatly enhanced blastocyst formation and pregnancy (Zhao et al., 2009). In vitro matured oocytes were provided by ART Inc. (Madison, WI). Somatic cell nuclear transfer (SCNT) was performed as previously described (Zhao et al., 2009). Briefly, 40 h after maturation, oocytes with a polar body (MII) were enucleated by aspirating the polar body and MII chromosomes and a small amount of surrounding cytoplasm. A single fetal fibroblast cell with α-1,3-Galactosyltransferase knockout and hDAF transgene was injected into the perivitelline space and placed adjacent to the recipient cytoplasm. The fusion and activation of the karyoplast-cytoplast complexes were achieved with 2 DC pulses of 1.2 kV/cm for 30 µsec from a BTX Electro Cell manipulator 2001 in fusion media (0.3 M mannitol, 1.0 mM CaCl2, 0.1 mM MgCl2, and 0.5 mM HEPES, Ph 7.3). The embryo culture media was Porcine Zygote Medium-3 (PZM-3) supplemented with 3 mg/ml BSA. Stock solutions of scriptaid were dissolved in dimethyl sulfoxide at 1 mM, and stored at -20 ºC. Stock solutions of MG132 were made in 100% ethanol at 10 mM and stored at -80 ºC. Immediately after electrofusion and activation, SCNT oocytes were treated with 0, 1, or 10 µM MG132 (0, 1, and 10 MG132 + Scriptaid) for 2 h and then treated with 500 nM scriptaid for another 16 h. Embryos were then washed and further cultured in PZM-3 until Day 7. Cleavage rate, blastocyst formation, and cell number were evaluated on Days 2 and 7, respectively, with the day of SCNT designated as Day 0.

Objective

To determine the effects of combined MG132 and Scriptaid treatment after electrical activation on early development of reconstructed embryos by SCNT.

Materials and Methods

1. Reconstruction of Embryos by SCNT

In vitro matured oocytes were provided by ART Inc. (Madison, WI). Somatic cell nuclear transfer (SCNT) was performed as previously described (Zhao et al., 2009). Briefly, 40 h after maturation, oocytes with a polar body (MII) were enucleated by aspirating the polar body and MII chromosomes and a small amount of surrounding cytoplasm. A single fetal fibroblast cell with α-1,3-Galactosyltransferase knockout and hDAF transgene was injected into the perivitelline space and placed adjacent to the recipient cytoplasm. The fusion and activation of the karyoplast-cytoplast complexes were achieved with 2 DC pulses of 1.2 kV/cm for 30 µsec from a BTX Electro Cell manipulator 2001 in fusion media (0.3 M mannitol, 1.0 mM CaCl2, 0.1 mM MgCl2, and 0.5 mM HEPES, Ph 7.3). The reconstructed embryos were then cultured in vitro.

2. Post-activation Treatment and Embryo Culture

The embryo culture media was Porcine Zygote Medium-3 (PZM-3) supplemented with 3 mg/ml BSA. Stock solutions of scriptaid were dissolved in dimethyl sulfoxide at 1 mM, and stored at -20 ºC. Stock solutions of MG132 were made in 100% ethanol at 10 mM and stored at -80 ºC. Immediately after electrofusion and activation, SCNT oocytes were treated with 0, 1 or 10 µM MG132 (0, 1, and 10 MG132 + Scriptaid) for 2 h and then treated with 500 nM scriptaid for another 16 h. Embryos were then washed and further cultured in PZM-3 until Day 7. Cleavage rate, blastocyst formation, and cell number were evaluated on Days 2 and 7, respectively, with the day of SCNT designated as Day 0.

Table 1. Cleavage rate, blastocyst formation rate, and cell number in SCNT embryos

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cleavage rate (%)</th>
<th>Blastocyst formation rate (%)</th>
<th>Cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 MG + Scriptaid</td>
<td>107</td>
<td>58.1 ± 7.5</td>
<td>18.5 ± 4.6ab</td>
<td>25.5 ± 2.1</td>
</tr>
<tr>
<td>1 MG + Scriptaid</td>
<td>110</td>
<td>62.7 ± 7.5</td>
<td>25.1 ± 4.6a</td>
<td>25.7 ± 1.9</td>
</tr>
<tr>
<td>10 MG + Scriptaid</td>
<td>111</td>
<td>62.5 ± 7.5</td>
<td>12.9 ± 3.3b</td>
<td>30.6 ± 2.4</td>
</tr>
</tbody>
</table>

a,b: means with different superscripts differ (P<0.05)

Results

A total of 328 oocytes/embryos, representing 8 replicates, were reconstructed using α-1,3-Galactosyltransferase knockout hDAF transgenic pig fetal-derived fibroblast cells. There was no difference in percent cleavage, or cell number among the three groups (Table 1). Interestingly, while there was no difference in the percent blastocyst between 1 µM and 0 µM MG132 treatment groups, more oocytes from the 1 µM MG132 group developed into blastocysts than in 10 µM MG132 (P<0.05).

Table 1. Cleavage rate, blastocyst formation rate, and cell number in SCNT embryos

Interpretation and future studies

The combined treatment of 1 µM MG132 and 500 nM Scriptaid had a beneficial effect on blastocyst formation of SCNT embryos. To determine the true developmental competence of these embryos, we are transferring these embryos to surrogate gilts.

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References


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