Preimplantation Embryo Development is Dependent on Glycine Metabolism

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Introduction

Recently a metabolic profiling of 60 cancer cell lines identified glycine as a crucial amino acid in cancer cell proliferation (Jain et al., 2012). They found that glycine consumption rates correlated with the speed of cell division. Jain and coworkers (2012) also looked at the expression of 1425 enzymes and found that enzymes in the mitochondria that are required for biosynthesis of glycine were highly correlated with the more proliferative cells. Another study conducted by Zhang and coworkers (2012) found that glycine metabolism and the enzyme glycine decarboxylase (GLDC) drive tumor-initiating cells. Glycine decarboxylase is a key component of the highly conserved glycine cleavage system in amino acid metabolism that catalyzes the breakdown of glycine to form CO₂, NH₃, and 5,10-methylene-tetrahydrofolate (CH₂-THF) to fuel one-carbon metabolism. Zhang and coworkers (2012) found that over-expressing GLDC alone, and other glycine/serine enzymes, promotes cellular transformation both in vitro and in vivo. Using metabolic analysis, it was shown that GLDC over-expression induces a dramatic increase in glycolysis and glycine metabolism, leading to changes in pyrimidine metabolism for cancer cell proliferation.

A previous transcriptional profiling database of in vivo fertilized and then on day 2 flushed and cultured in Porcine Zygote Medium 3 (PZM3) (IVC) compared to in vivo fertilized and in vivo cultured (IVV) until day 6 found that the glycine transporter, SLC6A9, was found to be up-regulated in embryos cultured in vitro compared to in vivo by deep sequencing. Three genes involved with glycine metabolism were found to be decreased in IVC embryos. The down-regulation of glycine cleavage enzyme transcripts could be due to a glycine deficiency in vitro. Two genes involved with glycine to serine metabolism were up-regulated in IVC compared to IVV.

Research Design and Methods

In Vitro Maturation, Fertilization, and Culture

Cumulus-oocyte complexes were matured for 44 hours in M199 supplemented with EGF, FSH, and LH. Oocytes with a visible polar body were selected and fertilized in modified Tris Buffered Medium for 5 hours and then placed into one of five treatment groups (PZM-MU1 (PZM3 + 1.69 mM arginine) with 0 mM, 1 mM, 5 mM, 10 mM or 20 mM glycine) in each well of a four well Nunc dish in a humidified atmosphere with 5% CO₂ in air at 38.5°C. After the 28-30 hr culture, embryos were washed twice in Tris Buffered Medium for 5 hours and then placed into one of five treatment groups (PZM-MU1 + G) in each well of a four well Nunc dish in a humidified atmosphere with 5% CO₂ in air at 38.5°C. After the 28-30 hr culture, embryos were stained with bisbenzimide (Hoechst 33342; Sigma) for 30 minutes then viewed under UV light. The results were analyzed using the MIXED procedure in SAS.

To determine the effect glycine had on development, the percent of embryos that developed to the blastocyst stage was analyzed using PROC GENMOD in SAS (SAS Institute, Cary, NC). A least significant difference post test comparison was completed to determine if significant differences existed between treatment groups. To acquire total cell number for each treatment, embryos were stained with bisbenzimide (Hoechst 33342; Sigma) for 30 minutes then viewed under UV light. The results were analyzed using the MIXED procedure in SAS.

The final culture experiment was completed to determine if glycine is acting as an osmolyte. Zygotes were cultured in either PZM-MU1 or PZM-MU1 + 10 mM glycine at either 260 mOsm, 275 mOsm, or 300 mOsm. Only one rep is completed so further analysis needs to be done.

Figure 3. Total cell number of blastocysts cultured in different concentrations of glycine

N=3 n=20, 22, 23, 27, 21, 15 (a,b,c,dP<0.05)

Figure 4. Representative blastocysts cultured in different glycine concentrations

Table 1: Glycine Related Genes

<table>
<thead>
<tr>
<th>Glycine Related Genes</th>
<th>IVC</th>
<th>IVV</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Solute carrier family member 6 (SLC6A9)</td>
<td>46.6</td>
<td>1.75</td>
<td>0.003</td>
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<tr>
<td>Glycine dehydrogenase (GLDC)</td>
<td>349</td>
<td>757</td>
<td>0.04</td>
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<tr>
<td>Amino oxidase, glycine cleavage T (AMT)</td>
<td>128</td>
<td>248</td>
<td>0.02</td>
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<tr>
<td>Glycine cleavage system protein H (GCSH)</td>
<td>564</td>
<td>1047</td>
<td>0.13</td>
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<tr>
<td>Phosphoserine phosphatase 1 (PSPH)</td>
<td>61.3</td>
<td>1.5</td>
<td>0.003</td>
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<tr>
<td>Phosphoserine amidotransferase (PSAT1)</td>
<td>188.5</td>
<td>1.1</td>
<td>0.055</td>
</tr>
</tbody>
</table>

Figure 1. Transcripts found by deep sequencing IVC and IVV day 6 blastocysts

Results

- Glycine had no effect on blastocyst percentage
- Cell number was significantly increased in d6 blastocysts cultured in 10 mM glycine
- It doesn’t appear that glycine is acting as an osmolyte
- More experiments need to be completed to assess the role glycine is playing in embryo development and to elucidate specific molecular mechanisms

Conclusions

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- Cell number was significantly increased in d6 blastocysts cultured in 10 mM glycine
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- More experiments need to be completed to assess the role glycine is playing in embryo development and to elucidate specific molecular mechanisms

References

Jain M et al., Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science 2012; 336:1040-1044.