Involvement of Na/K-ATPase in the differentiation of trophoblast

Are endogenous cardiotonic steroids responsible of trophoblast impairment associated with preeclampsia?

M. Podrecca*, V. Delsinne*, A. Bagrov,** H. Alexandre & J.-M. Colet*

*Department of Human Biology and Toxicology, Faculté de Médecine et de Pharmacie, Université de Mons, Belgium
**Department of Cardiovascular Science, National Institute on Aging, Baltimore, USA

Introduction

Permanent trophoblast stem cell lines (TS) were isolated from mouse blastocysts and cultured in a medium complemented with FGF4 and TGFβ1 (Tanaka et al., 1998; Erelbacher et al., 2004). Removing these factors initiates the differentiation of TS into Giant Trophoblast Cells (GTC). Epithelial to mesenchymal transition (EMT) together with DNA endoreplication are the two main features of this differentiation.

Although the mechanisms underlying EMT are still poorly understood, it was recently reported that Na/K-ATPase is involved in both maintenance of cell polarity and acquisition of cell motility (fig. 1). New findings on preeclampsia point out the Na/K-ATPase as a possible mediator of the differentiation of trophoblast. This disorder occurs only during pregnancy and affects both the mother and the conceptus (5-8% of all pregnancies, 20% of fetal and pregnancy associated mortality). It is a rapidly progressive condition characterized by maternal high blood pressure, proteinuria and defect in the invasion of trophoblast (Bellamy, 2007; Hladunewich, 2007). The key feature of this pathology is an abnormally elevated concentration of endogenous inhibitors of Na/K-ATPase (e.g. marinobufagenin and ouabain like molecule; also called cardiotonic steroids or CTS; Bagrov, 2008). Their possible effects on the conceptus have never been investigated and represent the aim of this project.

Methodology and first results

In vitro models

The effects of CTS on trophoblast invasion/differentiation will be firstly evaluated in vitro, using mice TS cells (a) and mice blastocyst’s outgrowths (c). The differentiation of GTCs (b, c – arrow) in presence or absence of CTS will be appreciated by the detection of several markers (fig. 2) and by the measurement of their invasive behaviour (fig. 3). Characterisation of GTC’s subpopulations will also be performed (fig. 4).

In vivo models

The second step of this PhD project is to evaluate in vivo the inhibition of Na/K-ATPase during pregnancy in mice. Morphological investigations will be combined with the analysis of biofluids by H-NMR and compared with analysis made in pregnant, normal, preeclamptic and previously preeclamptic women.

Morphological analysis

Histomorphometry

MALDI imaging mass spectrometry

H-NMR (blood, urine)

? Difference between normal and preeclamptic women?

? Early biomarkers of preeclampsia?

? Predisposition for preeclampsia?

References

We would like to thank Dr. J. Rosett for kindly providing TS cell line (EKE-3-5-GFP).

Acknowledgements

Figure 1. Involvement of Na/K-ATPase in signalling pathways controlling cell polarity and motility acquisition

Figure 2. We previously describe for the first time the localisation and expression of Na/K-ATPase in parietal GTCs and several markers associated with EMT (e.g. Na/K-ATPase α1 and E-Cadherin) during the differentiation of TS cells in GTCs (from d0 to d6). A strong correlation with their roles in cell polarity and motility acquisition (fig 1) was observed. The inhibition of the sodium pump by CTS and mRNAs will be evaluated using the same confocal microscopy techniques. The eventual impact of the ICM will be evaluated using blastocyst’s outgrowths.

Figure 3. Quantitative evaluation of growth, proliferation and motility of TS cells and GTCs will be performed by computer assisted videomicroscopy.

Figure 4. In addition to classical investigations (Simmons, 2007), GTC’s subpopulations will be characterized by High Resolution MAS H-NMR and H-NMR spectrometry of media, before and after inhibition of Na/K-ATPase. Thus, we could expect to point out specific biomarkers associated with preeclampsia and invasiveness.

Table 1. Specific markers expressed or not (green or red) in different subpopulations of GTC’s.

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>a1</th>
<th>b1</th>
<th>c1</th>
<th>d1</th>
<th>e1</th>
<th>f1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth</td>
<td>Green</td>
<td>Red</td>
<td>Green</td>
<td>Red</td>
<td>Green</td>
<td>Red</td>
</tr>
<tr>
<td>Proliferation</td>
<td>Green</td>
<td>Red</td>
<td>Green</td>
<td>Red</td>
<td>Green</td>
<td>Red</td>
</tr>
<tr>
<td>Invasion</td>
<td>Green</td>
<td>Red</td>
<td>Green</td>
<td>Red</td>
<td>Green</td>
<td>Red</td>
</tr>
</tbody>
</table>

Specific markers expressed or not (green or red) in different subpopulations of GTC’s.