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; Erelbacker *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 points 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, proteinury 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.

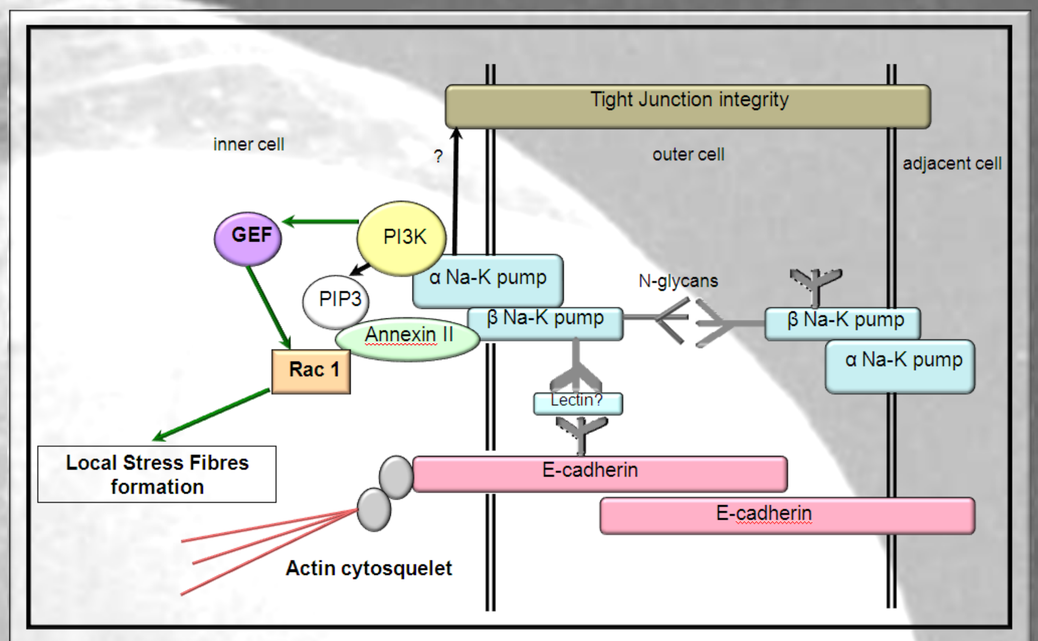


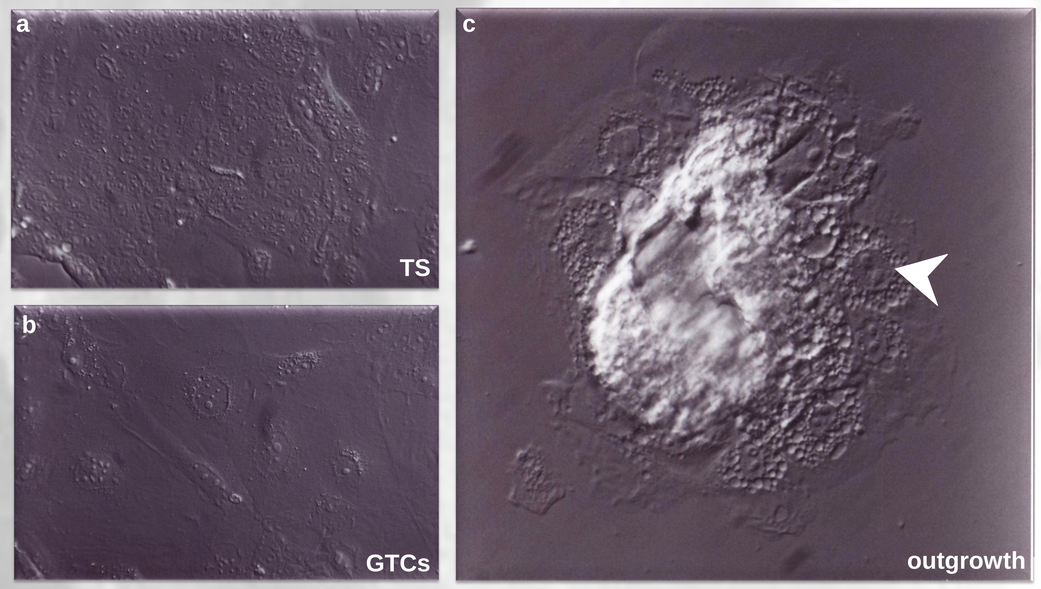
Figure 1. Involvement of Na/K-ATPase in signalling pathways controlling cell polarity and motility acquisition

(Rajasekaran, 2001, 2003 ; Barwe, 2005 ; Soshani, 2005 ;Vagin, 2006)

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).



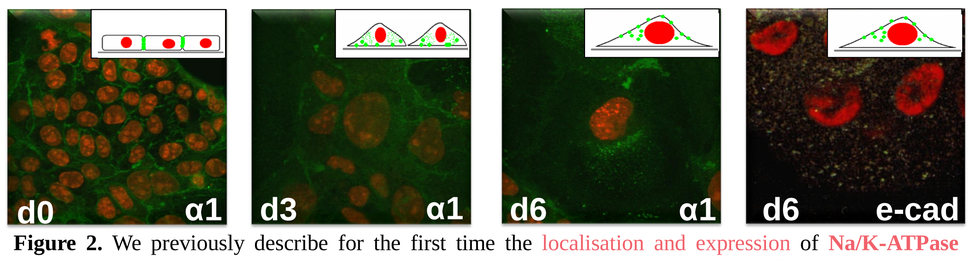


Figure 2. We previously describe for the first time the localisation and expression of **Na/K-ATPase isoforms** and several **markers associated with EMT** (e.g. Na/K-ATPase α1 and E-Cadherin) during the differentiation of TS cells in GTCs (from d0 to d6). A strong correlation with their roles in cell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

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.

Pregnant mice treated or not with marinobufagenin / ouabain like molecule (IV).

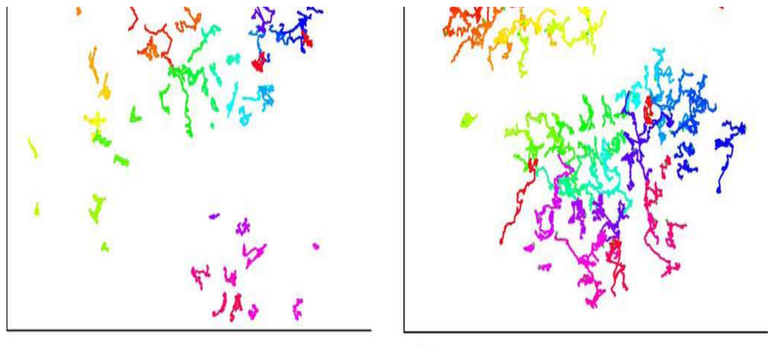
(previously) preeclamptic / pregnant / normal women

Morphological analysis
- ⇒ Histochemistry
- ⇒ MALDI imaging mass spectrometry

H-NMR (blood, urine, uterine mucus)
- ⇒ ? link between blood / urine / intrauterine concentration of CTS ? (relevance of *in vitro* observations)
- ⇒ ? early biomarkers of preeclampsia ?

H-NMR (blood, urine)
- ⇒ ?Difference between preeclamptic and pregnant women? ? early biomarkers of preeclampsia ?
- ⇒ ?Difference between normal and previously preeclamptic women? ? predisposition for preeclampsia ?

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



Monitoring of cells by computer asisted videomicroscopy, in presence or absence of an anti-migratory substance (respectively A and B; Hayot, 2006).

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**.

	Ctsq	Plf	Pl1	Pl2
sinusoidal GTCs				
canal GTCs				
spiral arteries associated GTCs				
parietal GTCs				

Specific markers expressed or not (green or red) in different subpopulations of GTCs (Simmons 2007). Ctsq: cathepsin Q, Plf: proliferin, Pl1: placental lactogen 1, Pl2: placental lactogen 2.

Acknowledgements

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

References

Bagrov A. Y. & J. I. Shapiro, 2008. Endogenous digitalis: pathophysiologic roles and therapeutic applications, *Nat. Clin. Pract. Nephrol.*, 4(7):378-392. **Barwe S. P. *et al.*, 2005.** Novel role for Na,K-ATPase in phosphatidylinositol 3-kinase signaling and suppression of cell mobility, *Molecular Biology of the Cell*, 16:1082-1094. **Bellamy L. *et al.*, 2007.** Preeclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis, *BMJ*, 335:974-979. **Erelbacher A. *et al.*, 2004.** Maintenance of trophoblat stem cell proliferation by TGFβ/activin, *Developmental Biology*, 275 :158-169. **Hayot C. *et al.*, 2006.** Characterizationof the activities of actin-affecting drugs on tumor cell migration, *Toxicology and Applied Pharmacology*, 211:30-40. **Hladunewich M. *et al.*, 2007.** Pathophysiology of the Clinical Manifestations of Preeclampsia, *Clin. J. Am. Soc. Nephrol.*, 2:543-549. **Rajasekaran S. A. *et al.*, 2001.** Na,K-ATPase activity is required for formation of tight junctions, desmosomes and induction of polarity in epithelial cells, *Molecular Biology of the Cell*, 12: 3717-3732. **Rajasekaran A. K. *et al.*, 2003.** Role of Na-K-ATPase in the assembly of tight junctions, *Am. J. Physiol. Renal Physiol.*, 285: 388-396. **Simmons D. G. *et al.*, 2007.** Diverse subtypes and developmental origins of trophoblast giant cells in the mouse placenta, *Developmental Biology*, 304(2):567-578. **Shoshani L. *et al.*, 2005.** The polarized expression of NaK ATPase in epithelia depends on the association between β-subunits located in neighboring cells, *Molecular Biology of the Cell*, 16: 1071-1081. **Tanaka S. *et al.*, 1998.** Promotion of Trophoblast Stem Cell Proliferation by FGF4, *Science*, 282: 2072-2075. **Vagin O. *et al.*, 2006.** The role of the β1 subunit of the Na,K-ATPase and its glycosylation in cell-cell adhesion, *The journal of biological chemistry*, 281/51:39573-39587.