Article

Does altered oxygenation or reactive oxygen species alter cell turnover of BeWo choriocarcinoma cells?

Dr Alexander Heazell is a Clinician Scientist at the University of Manchester, UK. His primary research interest is the control of placental cell turnover in normal and abnormal pregnancies. He is particularly interested in the role of the placenta in the development of pre-eclampsia and intra-uterine growth restriction.

Dr Alexander Heazell

Alexander EP Heazell[1,2], Natalie NJ Taylor[1], Susan L Greenwood, Philip N Baker, Ian P Crocker
Maternal and Fetal Health Research Group, University of Manchester, St Mary's Hospital, Manchester, UK
[1]AEPH and NNJT contributed equally to the manuscript.
[2]Correspondence: Tel: +44 161 2765460; Fax: +44 161 2766134; e-mail: alex_heazell@talk21.com

Abstract

This study assessed the effect of 20 and 6% ambient oxygen (O_2) or 5–50 µmol/l hydrogen peroxide (H_2O_2) on apoptosis, necrosis, proliferation and fusion of BeWo cells. The expression of p53, Mdm2 and Bax was assessed by western blotting. Apoptosis was increased in cells cultured in 6% O_2 tension and 50 µmol/l H_2O_2 ($P < 0.05$, $P < 0.01$ by ADP:ATP ratio). In the same conditions, cell viability as estimated by the MTT assay was decreased (6% O_2 $P < 0.01$, 50 µmol/l H_2O_2 $P < 0.05$). Human chorionic gonadotrophin secretion was decreased by culture in 6%O_2 and 50 µmol/l H_2O_2 ($P < 0.05$). Cell fusion was also decreased by treatment with 50 µmol/l H_2O_2 ($P < 0.05$). Treatment with 50 µmol/l H_2O_2 was associated with increased expression of p53 and decreased expression of Mdm2 ($P < 0.05$). This study provides evidence that BeWo cell turnover is altered following exposure to hypoxia or ROS. It is concluded that BeWo cell culture is an appropriate model for investigating the regulation of trophoblast cell turnover. In addition, these data support a role for p53 in mediating altered trophoblast cell turnover in response to oxidative stress.

Keywords: *apoptosis, BeWo choriocarcinoma cell line, hypoxia, reactive oxygen species, trophoblast*

Introduction

Cell turnover is a tightly regulated event; in a tissue in a state of equilibrium a balance must be maintained between cell proliferation and cells lost by damage or death. In villous trophoblast, nuclei in the syncytiotrophoblast undergo morphological changes similar to apoptosis and some degenerate nuclei are gathered together in syncytial knots, which are then lost into the maternal circulation (Huppertz and Kingdom, 2004). The syncytiotrophoblast is maintained by proliferation and fusion of underlying cytotrophoblast cells (Huppertz and Kingdom, 2004). The equilibrium between maintenance and loss of syncytiotrophoblast is disturbed in severe early-onset pre-eclampsia and intrauterine growth restriction (IUGR), both of which are associated with increased apoptosis (Smith *et al.*, 1997; Leung *et al.*, 2001), increased formation of syncytial knots (Heazell *et al.*, 2006) and disordered proliferation (Macara *et al.*, 1996). Similar disruption in cell turnover has also been observed in cases of missed miscarriage (Hempstock *et al.*, 2003). Investigation of the regulation of apoptosis and proliferation in trophoblast may provide insights into the pathophysiology of these conditions.

The aberrant turnover of villous trophoblast in pre-eclampsia and IUGR is thought to result from exposure to hypoxia and/or oxidative stress, as severe pre-eclampsia and IUGR are associated with a failure of conversion of spiral uterine arteries from convoluted vessels to wide flaccid conduits required for the delivery of maternal blood to the placenta (Meekins *et al.*, 1994; Naicker *et al.*, 2003). It is postulated that this reduction in blood flow results in placental hypoxia or hypoxia–reperfusion injury. This is supported by evidence that placentas in pre-eclampsia show similar gene expression to placental explants cultured in hypoxic conditions (Soleymanlou *et al.*, 2005).

© 2009 Published by Reproductive Healthcare Ltd, Duck End Farm, Dry Drayton, Cambridge CB23 8DB, UK

Cell turnover is a tightly regulated process controlled by many proteins. In many cell types, apoptosis is induced by cell damage such as insults from hypoxia or oxidative stress. p53 is increased in response to different noxious stimuli including these conditions and has been termed the 'guardian of the genome', as damaged cells are destroyed (Prives and Hall, 1999). p53 is negatively regulated by Mdm2, which targets the p53 protein for destruction via the proteosome (Iwakuma and Lozano, 2003). p53 promotes the transcription of Mdm2 providing a negative feedback loop preventing unwanted apoptosis in healthy cells. The balance of p53 and Mdm2 is essential in determining cell survival (de Rozieres *et al*., 2000). p53 promotes transcription of downstream pro-apoptotic factors such as Bax (Miyashita and Reed, 1995). These factors are present in villous trophoblast at the mRNA and protein level, indicating that they may have a role in regulating cell turnover (Fulop *et al*., 1998; Qiao *et al*., 1998; Allaire *et al*., 2000). Preliminary data suggest that expression of p53 is increased in villous trophoblast of pregnancies complicated by IUGR (Levy *et al*., 2002) and following exposure to hypoxia (Levy *et al*., 2000; Heazell *et al*., 2008).

Various in-vitro models have been used to investigate the effects of altered oxygenation and ROS on trophoblast cell turnover including: culture of whole placental tissue, isolated primary trophoblast and choriocarcinoma cell lines. Apoptosis may be induced *in vitro* in placental explants following exposure to reactive oxygen species (ROS) (Moll *et al*., 2007), hypoxia–reoxygenation injury (Hung *et al*., 2002) or hypoxia (Heazell *et al*., 2008). Hypoxia also induces apoptosis in isolated cytotrophoblasts (Levy *et al*., 2000). In accordance with other in-vitro models, BeWo choriocarcinoma cells undergo proliferation, followed by fusion to form multinucleate syncytia, thereby mirroring the formation of syncytiotrophoblast *in vivo*. As a result, BeWo cells have been used to investigate some aspects of trophoblast cell turnover, including fusion and formation of syncytia, apoptosis and proliferation (Kudo *et al*., 2003b; Al-Nasiry *et al*., 2006; Bae *et al*., 2007; Hu *et al*., 2007). Despite several investigations of the effects of oxygen (O_2) on fusion and nutrient transport by BeWo cells, there has been no investigation of the effects of O_2 or ROS on apoptosis, which may add to understanding of common pregnancy complications. It was postulated here that exposing BeWo cells to reduced O_2 tension or ROS in the form of hydrogen peroxide (H_2O_2) would alter cell turnover including: apoptosis, proliferation and cell fusion. In addition, it was postulated that changes in cell turnover would be associated with altered expression of p53, Mdm2 and Bax.

Materials and methods

Unless otherwise stated, reagents were obtained from Sigma-Aldrich Chemical Company (Poole, Dorset, UK).

Cell culture

BeWo cells were obtained from the European Collection of Animal Cell Cultures (Porton Down, Wiltshire, UK) and had been stored in liquid nitrogen. For experimentation, cells were rapidly thawed using Dulbecco's modified Eagle's medium-F12 supplemented with 10% fetal bovine serum, 30 mg/l penicillin, 50 mg/l streptomycin and 146 µg/l glutamine warmed to 37°C and transferred to a 75 cm^3 flask and grown to confluence. When confluent, cells were washed with warm phosphate-buffered saline (PBS), and treated with 5 ml 0.05% trypsin–EDTA solution for 2 min. Cells were collected in 10 ml of warmed culture medium and counted using a haemocytometer (Marienfeld, Germany). Cells were then seeded into either 6-well plates (1.5×10^6/well) or 96-well plates (10–50 $\times 10^5$/well) for subsequent culture (Corning Inc., NY, USA). Cells were cultured for 48 h in 20% ambient O_2 (control). To assess the effects of reduced oxygenation cells were cultured for 48 h in 6% atmospheric O_2 for 48 h or in 20% O_2 for 24 h followed by 6% O_2 for 24 h. The effect of ROS was investigated by culture in 20% O_2 for 24 h, then 5, 10 or 50 µmol/l H_2O_2 was added and cells were cultured for a further 24 h (**Figure 1**).

Assessment of apoptosis

Apoptosis was assessed using two methods. Firstly, by the Apopercentage kit according to the manufacturers' instructions (Biocolor, Northern Ireland, UK). The details of reagents used for this method are under patent and cannot be disclosed. Briefly, cells were cultured on 96-well plates at a density of 5×10^5/well. Eight wells were cultured per experimental condition for five separate passages ($n = 5$). After the treatment period, the culture medium was replaced with medium containing dye, which is taken up by apoptotic cells. The cells were returned to their culture environment for a further 30 min. Cells were then gently washed with warmed PBS and photographs taken using an inverted microscope (Leica, Germany). Cells were then treated with dye release reagent for 10 min at room temperature and absorbance read at 550 nm using a spectrophotometer (Molecular Devices, Wokingham, UK). Apoptosis was also assessed using a commercially available ADP:ATP ratio kit (Apoglow kit; Cambrex, Verviers, Belgium) with modifications to the manufacturers' instructions. This method has been previously used on primary trophoblast culture (Crocker *et al*. 2003). Briefly, BeWo cells were cultured in a white walled 96-well plate at a density of 2×10^5/well; eight wells were cultured per experimental condition for five separate passages ($n = 5$). Following culture, cells were treated to release ADP and ATP, then combined with a luciferin reagent, which releases light in the presence of ATP. A reading was taken immediately using a luminometer (Molecular Devices) (reading A) and the light emission was allowed to decay for 20 min. A further reading was then taken (reading B) and ADP-converting reagent added, and a final reading taken after 2 min (reading C). The ADP:ATP ratio was calculated by (reading C–reading B)/reading A.

Assessment of necrosis

Necrotic cell death was quantified using a commercially available lactate dehydrogenase (LDH) kit, which measures the reduction of a tetrazolium to formazan salt in the presence of NADH, which is generated by the conversion of lactate to pyruvate by LDH (Roche Applied Science, Lewes, UK). Conditioned culture media was defrosted on ice and combined with the reagents as per manufacturer's instructions. Unconditioned culture medium was used as a negative control. Absorbance was measured at 490 nm (Molecular Devices). The coefficient of variation for this assay was 5.97%.

Assessment of mitochondrial viability

The reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a formazan salt was used as a measure of cell viability as previously described (Al-Nasiry

Download English Version:

https://daneshyari.com/en/article/3972286

Download Persian Version:

https://daneshyari.com/article/3972286

Daneshyari.com