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Original Article

Oxygen and glucose dependent viability of HLA-G positive and negative trophoblasts using ACH-3P cells as first trimester trophoblast-derived cell model☆

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ABSTRACT

Aims: During pregnancy HLA-G negative proliferative trophoblasts at the tips of anchoring villi form cell columns from which HLA-G positive extravillous trophoblasts invade maternal tissues. During the first trimester of pregnancy an oxygen gradient ranges from placental low to decidual high oxygen, which may have a differential impact on survival of the two trophoblast subpopulations. Moreover, diabetes-associated hyperglycemia may also influence trophoblast proliferation.

Methods: ACH-3P cells were separated by magnetic beads into HLA-G positive and negative cells and checked by PCR and Western blotting. Cell cultures were performed under varying oxygen and glucose concentrations. Numbers of viable and dead cells were assessed and used to calculate proliferation rates.

Results: After separation, HLA-G positive and negative first trimester trophoblast-derived ACH-3P cells exhibit fewer viable cells under hyperglycemia at 2.5% and 8% oxygen, while at 21% oxygen no viable cells were detectable. Cell numbers of HLA-G negative cells were higher compared to HLA-G positive cells at 2.5% and 8% oxygen, while there were significantly less cells at 8% compared to 2.5% only in HLA-G positive cells.

Conclusion: We conclude that the separated cell types are sensitive to both oxygen and glucose independent from each other. Furthermore, oxygen may be one regulator to reduce proliferation of invading HLA-G positive trophoblasts, while alterations in the oxygen gradient early in pregnancy may have deleterious effects on the number of invading extravillous trophoblasts.

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1. Introduction

During the first trimester of pregnancy proliferative cytotrophoblasts develop cell columns at the tips of anchoring villi. The post-proliferative daughter trophoblasts differentiate towards invasive extravillous trophoblasts and invade uterine tissues.[1,2] As a consequence of differentiation extravillous trophoblasts change their phenotype and start to express the human leukocyte antigen-G (HLA-G).[3,4]

The non-classical HLA-G molecule only shows a very low degree of allelic variants and thus clearly differs from classical HLA class 1 molecules. Being primarily expressed by extravillous trophoblast, HLA-G acts to modulate the maternal immune response and leads to tolerance by influencing cells of adaptive as well as innate immunity such as B- and T-cells, macrophages and NK cells.[5,6] Soluble HLA-G release by extravillous trophoblasts may damp potentially harmful immune responses not needed a direct cell-to-cell contact between trophoblast and maternal immune cell.[6] This is only needed for those trophoblasts that come into direct contact to maternal immune cells, which is especially true for invading extravillous trophoblasts.

Onset of placental maternal blood flow is only established at the beginning of the second trimester.[7] Thus, early in pregnancy the placenta and embryo develop in a low oxygen environment. The pO_2 within the placenta prior to the onset of maternal blood towards the placenta is about 20 mm Hg, while at the same time the pO_2 within decidual tissues is about 60 mm Hg.[7] Diabetic pregnancies are characterized by impaired trophoblast invasion and inadequate remodeling of spiral arteries predisposing them to a variety of pregnancy pathologies.[8,9]

Based on our recent investigation that oxygen modulates first trimester trophoblast response on glucose,[10] the present study tested the hypothesis that HLA-G positive and negative trophoblasts differ in their proliferative response to oxygen and glucose. We used a recently generated first trimester trophoblast-derived cell line, ACH-3P, which was established by fusion of a human choriocarcinoma cell line (AC1-1) and isolated first trimester trophoblasts (week 12).[11] ACH-3P cells represent both subpopulations, HLA-G negative villous as well as HLA-G positive extravillous cytotrophoblasts. Here we show that HLA-G negative ACH-3P cells have a better survival rate than HLA-G positive cells under 2.5% and 8% oxygen, independent of the glucose level.

2. Materials and methods

2.1. Cell culture

ACH-3P (Fig. 1) cells were cultured at 37 °C, 5% CO_2 and 21% O_2 in 175 cm^2 flasks (Nunc, Roskilde, Denmark) in DMEM containing low D(+)-glucose, sodium pyruvate and L-glutamine (Gibco, Invitrogen Ltd, Paisley, UK). Medium was supplemented with 10% heat-inactivated FCS (FCS; Gibco, Invitrogen Ltd., Paisley, UK) and 100 units/ml penicillin/100 μg/ml streptomycin (PAA Laboratories; Pasching, Austria). Cells were grown in 75 cm^2 flasks (5×10^5 cells/flask; Nunc), 6 well dishes (5×10^4 cells/well; Iwaki, Tokyo, Japan) or 24 well dishes (1×10^4 cells/well; Corning, Lowell, USA). The day before any treatment, medium was replaced with fresh DMEM with low D(+)-glucose, without FCS, supplemented with 1% penicillin/streptomycin over night. On the following day, cells were treated with DMEM containing low (normoglycemia; 5.5 mmol/l) or high (hyperglycemia; 25 mmol/l) D(+)-glucose, supplemented with 2% FCS and 1% penicillin/streptomycin, for up to 3 day at 37 °C, 5% CO_2 and 2.5%, 8% or 21% oxygen in an XVIVO incubation system (model G300C; BioSpherix, NY, USA). 19.5 mmol/l L(−)-glucose was added to DMEM low D(+)-glucose as osmotic control. Media were replaced every 24 h in the XVIVO incubation system without altering oxygen conditions.

2.2. Immunoseparation of HLA-G positive and negative cells

Cells were separated using magnetic beads (Dynabeads M-450, Invitrogen) coated with MEM-G/9. Cells were incubated with low glucose DMEM containing the antibody with agitation at 4 °C. Subsequently, a Dynal magnet (Invitrogen) was used to separate HLA-G positive and negative cells. Cells were washed and incubated over night. Separation quality was determined by semi-quantitative RT-PCR.

On the following day, 5×10^4 cells/well were cultured in DMEM without FCS over night, then treated with DMEM low (5.5 mmol/l) or high (25 mmol/l) glucose, supplemented with 2% FCS for 72 h at 37 °C, 5% CO_2 and 2.5%, 8% or 21% oxygen in an XVIVO incubation system (Model G300C, BioSpherix Ltd, Lacona, USA). The osmotic control contained 19.5 mmol/l L(−)-glucose added to low glucose DMEM.

2.3. Semi-quantitative RT-PCR

RT-PCR used the following primer pairs for HLA-G (Forward: AGG AGA CAC GGA ACA CCA AG; Reverse: GGA GAG CCT ACC TGG AGG) and an internal control RPL30 (Forward: CCT AAG GCA GGA AGA TGG TG; Reverse: AG TCT GTT CTG GCA TGC TT; MWG Biotech AG, Ebersberg, Germany). RNA was isolated using TRI Reagent (Applied Biosystems, Foster City, USA). Total RNA (200 ng) was used for the one-step RT-PCR kit (Qiagen, Germantown, USA). For HLA-G and RPL30 mRNA expression 29 and 24 cycles were utilized, respectively, in a thermo cycler Gene Amp® PCR System 9700 (Applied Biosystems) with an annealing temperature of 60 °C. Samples were loaded on a 2% agarose gel and band intensity was detected by Multimage III (Cell Bioscience, Santa Clara, USA). Gene expression was quantified with RPL30 using AlphaView software (Cell Bioscience).

2.4. Cell count and viability assay

Cell number and viability was determined by automatic cell counting (CASY® TT, Schärfe System, Reutlingen, Germany). Cells were enzymatically detached, cell suspensions were diluted (1:200) using the CASY buffer CASY®ton (Roche, Mannheim, Germany), and viable and dead cells were quantified using the CASY technology, which is an electric field multichannel cell counting system.