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SHORT COMMUNICATION

Trophoblast Invasion of Spiral Arteries: a Novel In Vitro Model

J. E. Cartwright[a,c], L. C. Kenny[b], P. R. Dash[a], I. P. Crocker[b], J. D. Aplin[b], P. N. Baker[b] and G. St J. Whitley[a]

[a] Department of Biochemistry and Immunology, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE and [b] Maternal and Fetal Health Research Centre, St Mary's Hospital, Whitworth Park, Manchester, M13 0JH

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Extravillous trophoblasts invade the uterine wall (interstitial invasion) and the spiral arteries (endovascular invasion), replacing the cells of the vessel wall and creating a high-flow low-resistance vessel. We have developed a novel model to allow the interactions between the invading trophoblast cells and the cells of the spiral artery to be directly examined. Unmodified (non-placental bed) spiral arteries were obtained from uterine biopsies at caesarean section. Fluorescently labelled trophoblasts were seeded on top of artery segments embedded in fibrin gels (to study interstitial invasion) or perfused into the lumen of arteries mounted on a pressure myograph (to study endovascular invasion). Trophoblasts were incubated with the vessels for 3–5 days prior to cryo-sectioning. Both interstitial and endovascular interactions/invasion could clearly be detected and a comparison of the extravillous trophoblast cell line, SGHPL-4 and primary first trimester cytotrophoblasts showed both to be invasive in this model. This novel method will prove useful in an area where in vitro studies have been hampered by the lack of suitable models directly examining cellular interactions during invasion.

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INTRODUCTION

Within the placenta in early gestation, trophoblasts differentiate and give rise to sub-populations of cells. One of these, the extravillous trophoblast, invades the uterine wall (interstitial invasion) and its blood vessels (endovascular invasion). During normal pregnancy, the trophoblasts destined to be endovascular adopt a more endothelial cell (EC)-like phenotype and invade the uterine spiral arteries as far as the myometrial segments. Immunohistochemical studies of placental bed biopsies suggest that trophoblasts and EC transiently coexist on the walls of partially modified spiral arteries (Pijnenborg et al., 1980; Zhou et al., 1997) where they migrate along the luminal surfaces of EC, invading the vessels and partially replacing the EC and most of the musculoelastic tissue in the vessel walls (Pijnenborg et al., 1980; Enders and King, 1991; Blankenship et al., 1993; Meekins et al., 1997). This creates a high-flow, low-resistance circulation that maximizes maternal blood flow to the placental villi at the maternal–fetal interface. There is contrasting evidence as to whether trophoblasts themselves are important in arterial remodelling. Although it has been suggested that some changes in the decidual vessels occur independently (Craven et al., 1998) as part of the maternal response to pregnancy, there is also strong evidence to suggest that invasive interstitial trophoblasts prepare the decidual spiral arteries for endovascular trophoblast migration (Pijnenborg et al., 1983; Blankenship and Enders, 1997; Kam et al., 1999). The invasive trophoblast may play an important role in inducing further changes either by interactions or factors produced by the interstitial trophoblast or by direct cellular interactions of the endovascular trophoblast with the cells of the vessel that they subsequently replace.

The pathogenesis of both pre-eclampsia (PE) and intrauterine growth restriction (IUGR) is associated with trophoblasts failing to adopt an EC-like phenotype and endovascular invasion failing to proceed beyond the superficial portions of the spiral arteries in early pregnancy (Meekins et al., 1994; Lim et al., 1997; Zhou et al., 1997) although Divers et al. (1995) failed to find any clear phenotypic differences. Since there is still abundant interstitial migration of trophoblasts in the placental bed (Pijnenborg et al., 1998), the ability of trophoblasts to enter and transform the spiral arteries appears to be a significant difference between normal and PE/IUGR pregnancies (Khong et al., 1986). The importance of interactions between trophoblasts and the vascular cells of the spiral arteries, which may account for these differences, have yet to be determined in normal or PE/IUGR pregnancies.

Studies of spiral arteries are confined primarily to immunohistochemical analysis of placental bed biopsies (Genbacev et al., 1996; Zhou et al., 1997; Lyall et al., 1999, 2000) while in vitro studies have been hampered by the lack of suitable models to directly examine cellular interactions during

[c] To whom correspondence should be addressed at: Department of Biochemistry and Immunology, St George's Hospital Medical School, Cranmer Terrace, London, SW17 0RE. Tel: 020 8725 0803; Fax: 020 8725 2992. E-mail: j.cartwright@sghms.ac.uk

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invasion. Here we present a novel in vitro model of spiral artery invasion and remodelling, developed using spiral artery explants, extravillous trophoblast cell lines and primary cytotrophoblasts.

MATERIALS AND METHODS

Trophoblast cultures

SGHPL-4 is a well-characterized cell line derived from primary human extravillous trophoblasts that retains many features of normal extravillous cells including expression of cytokeratin-7, BC-1, CD9, hPL, hCG, HLA-Class I and HLA-G (Choy and Manyonda, 1998; Cartwright et al., 1999; Choy et al., 2000; Shiverick et al., 2001). SGHPL-4 cells were cultured in Hams F10 containing 2 mM L-glutamine, 0.12 per cent (w/v) sodium bicarbonate, 16 μg/ml gentamicin and supplemented with 10 per cent (v/v) fetal calf serum (FCS).

Primary cytotrophoblasts were isolated from late first trimester placenta. Tissue (6 g) was washed thoroughly in Hanks' balanced salt solution, chopped with scissors then washed twice more. It was resuspended in trypsin (0.125 per cent) and DNase (1 mg/ml) and incubated for 35 min at 37°C. The cells were harvested and the enzymatic treatment repeated. Tissue pieces were removed by filtration (100 μm) and the trypsin was neutralized. Cells were loaded on a Percoll gradient and after centrifugation at 1800 *g* for 30 min, were collected from the 30–45 per cent range. Cells were pelleted, resuspended in serum-free medium (Hams F12/DMEM) and plated on Matrigel-coated flasks.

Fluorescent labelling of trophoblast cells

Trophoblasts (SGHPL-4 cells and primary cytotrophoblasts) were labelled with CellTracker Orange probe (Molecular Probes). Cells were incubated with normal medium containing 5 μM probe for 30 min at 37°C. The medium was replaced with fresh medium and incubated for another 30 min at 37°C.

Co-culture of trophoblasts and spiral artery explants

Myometrial biopsies were obtained from normal pregnant women ($n=5$) undergoing elective caesarean section at term for reasons such as breech presentation. Using a stereomicroscope, we have developed a technique for identifying and dissecting unmodified spiral arteries under sterile conditions. To facilitate the investigation of interstitial invasion, the arteries were isolated with a small amount of surrounding tissue present. For the investigation of endovascular invasion the arteries were dissected so that they consisted of only the EC layer and muscular tunica media [Figure 1(A)].

Interstitial invasion. Following dissection, the ends of segments of spiral artery were tied off and the arteries were immediately immobilized in fibrin gels. Fibrin gels were formed as previously described (Cartwright et al., 1999). Fluorescently labelled SGHPL-4 cells were resuspended at 10^5 cells/ml in EC medium (large vessel EC growth medium, TCS Biologicals) and seeded on top of the preparation (5×10^4 cells in total) and incubated for 5 days. We have previously shown that extravillous trophoblast cell lines will invade fibrin gels whilst remaining viable in this environment for >5 days (Cartwright et al., 1999; Lash et al., 1999).

Endovascular invasion. Isolated spiral arteries were mounted on a pressure myograph and the lumen of the spiral artery was perfused with fluorescently labelled trophoblasts at 5×10^6 cells/ml in EC medium (with approximately 5×10^4 cells perfused into each artery segment). The ends of each spiral artery segment were tied off (to prevent the cells diffusing out of the vessel) and the artery was then transferred to and supported in a fibrin gel with culture medium added on top and incubated for 3 days [Figure 1(C)].

Cryosectioning and fluorescent microscopy

The segments of spiral artery were removed from the fibrin gels, immediately placed in embedding compound, frozen at − 40°C and 5–10 μm cryostat sections cut. The invasion of the artery by trophoblast cells was determined by fluorescence microscopy. Nuclei were counterstained by the addition of Vectashield mounting medium with DAPI (Vector Laboratories). Digital images were captured using a cooled CCD camera and Image Pro-Plus software (Media Cybernetics).

RESULTS

CellTracker Orange-labelled trophoblasts (SGHPL-4 cells) invaded spiral artery segments embedded in fibrin gels [Figure 1(B)]. Interstitial invasion of cells can be seen, with red fluorescent cells visible within the tissue surrounding the vessel. Labelled trophoblasts perfused into the lumen of the artery to model the endovascular population could be seen lining the vessel [Figure 1(D)] or invading into the vessel wall [Figure 1(E–I)]. Red fluorescence within the vessel wall can be seen in bright field illumination [Figure 1(F–H)]. The red fluorescence is cell-associated as seen by colocalization with DAPI staining [Figure 1(I)]. Endovascular invasion was observed with both SGHPL-4 cells [Figure 1(E and F)] and primary cytotrophoblasts [Figure 1(G–I)].

DISCUSSION

Previous studies of the changes brought about by trophoblasts in maternal spiral arteries have relied on immunohistochemical

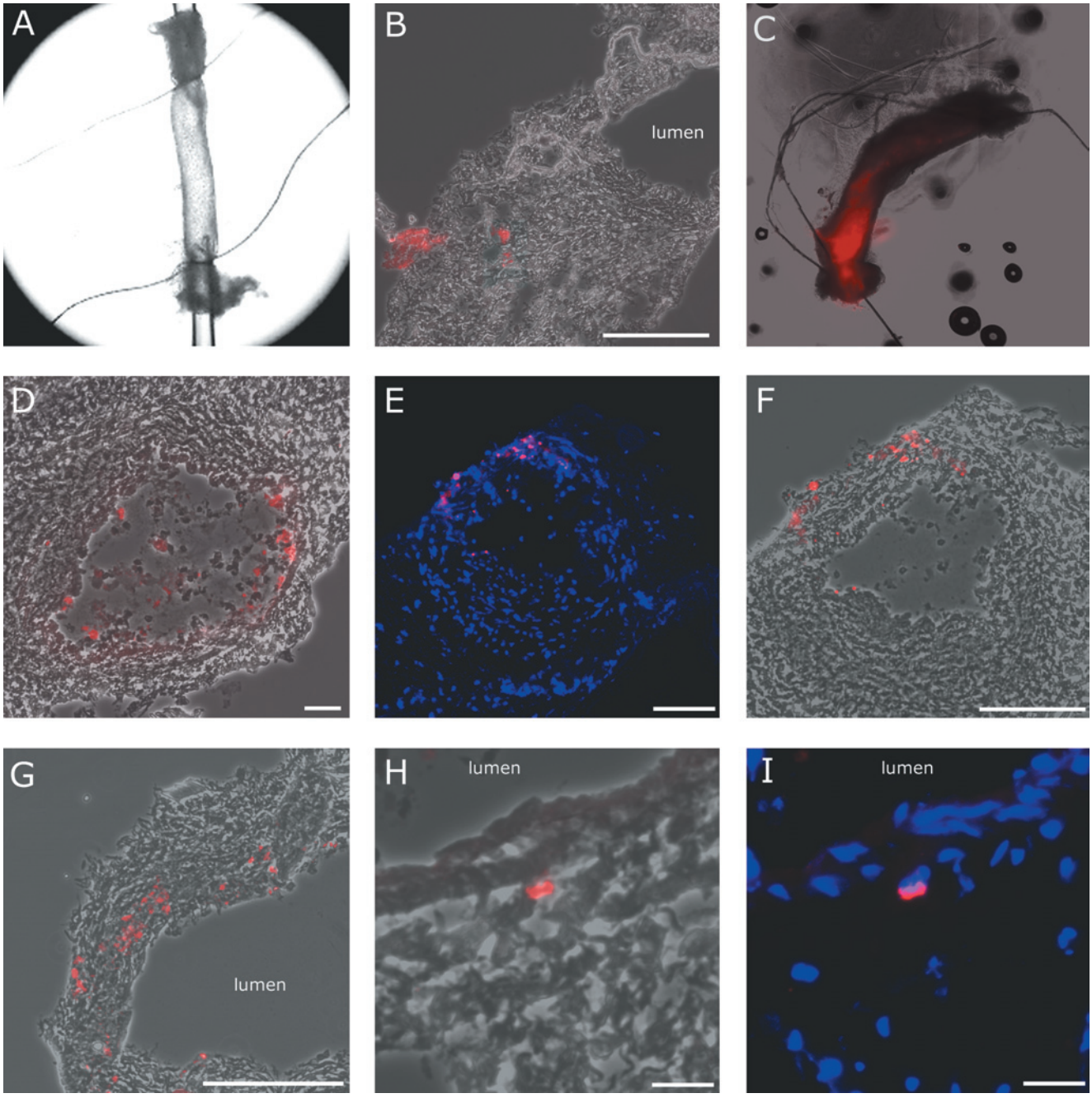

Figure 1. Trophoblast invasion of spiral artery explants. A: Spiral artery isolated from a non-placental bed site from a woman with a normal pregnancy and mounted and photographed under isobaric conditions. B: Interstitial invasion of spiral arteries by CellTracker Orange labelled-trophoblasts. Segments of spiral artery were isolated and embedded in fibrin gels. Labelled SGHPL-4 cells were added to the top of the fibrin gels in medium and were allowed to invade the vessels for 5 days. Frozen sections were taken and observed by fluorescence microscopy. Invasion by trophoblasts is indicated by patches of bright red fluorescence and can be seen within the tissue surrounding the vessel. C–I: Endovascular invasion of spiral arteries. Segments of spiral artery were isolated and mounted on a pressure myograph. Arteries were perfused with CellTracker Orange labelled-trophoblasts, the ends were tied off and the vessel was embedded in a fibrin gel. The vessels were incubated for 3 days prior to taking frozen sections. C: Labelled trophoblasts within the lumen of the vessel at the start of the experiment. D: Red fluorescent cells (labelled trophoblasts) can be seen lining and interacting with cells of the vessel. E–I: Endovascular invasion is also seen, with some cells migrating quite deep in the vessel wall. D, E and F: Labelled SGHPL-4 cells. G, H and I: Labelled primary cytotrophoblasts. I: Counterstaining with DAPI, which detects nuclei (blue fluorescence) determined that the red fluorescence was cell associated. Scale bars: 200 μm B, E, F; 35 μm; D; 100 μm G and 20 μm H and I.

analysis of placental bed biopsy sections. This is limited to staining for markers with appropriate spatio-temporal patterns of expression. The aim of this study was to establish whether trophoblasts interact with cells of the vessel wall and to establish a simple three-dimensional model that will enable changes that occur during the invasive process to be studied. It has been suggested previously that trophoblasts and EC transiently coexist on the walls of spiral arteries (Pijnenborg et al., 1980; Zhou et al., 1997), prior to remodelling the vessels and partially replacing the EC and most of the musculoelastic

tissue in the vessel wall (Pijnenborg et al., 1980; Enders and King, 1991; Blankenship et al., 1993; Meekins et al., 1997). It is clear from our results that trophoblasts are capable of interacting with the vessel. Cells are observed attached to the vessel surface and invading into the wall. Adhesive interactions between the trophoblasts and the vascular EC and the mechanisms that control entry of trophoblasts into the vessel wall can therefore be studied using this model. These data indicate that both SGHPL-4 cells and primary cytotrophoblasts are invasive. Since SGHPL-4 cells are amenable to stable genetic manipulation, specific targeting of proteins expressed by trophoblasts will allow the role of specific molecular pathways to be established. Determination of the effect of growth factors and other influences such as oxygen tension on invasion and cell-cell interactions, will also be facilitated. The opportunity to study both interstitial and endovascular invasion is particularly interesting as it has been suggested that invasive interstitial trophoblasts prepare the spiral arteries for endovascular trophoblast migration (Pijnenborg et al., 1983; Blankenship and Enders, 1997; Kam et al., 1999).

Determining how trophoblasts interact with cells of the maternal vessels would represent a major advance in our understanding of the adaptation to pregnancy and may provide insight into diseases characterized by poor invasion such as PE and IUGR. This novel in vitro model is equally applicable to the study of complicated pregnancies since invasion of non-placental bed spiral arteries from PE and IUGR pregnancies can be compared to arteries from normal pregnancies at similar gestational age.

In these studies we have used arteries obtained at term Caesarian sections since we aim to compare normal with PE/IUGR pregnancies. However the comparison between first trimester and term non-placental bed spiral arteries would also be of great interest since gestational differences in the spiral arteries may well influence how the trophoblasts invade.

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