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Hemangioblastic foci in human first trimester placenta: Distribution and gestational profile

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ABSTRACT

Introduction: The human placenta is a site of both hematopoiesis and vasculogenesis. There are reports of hemangioblastic foci (HAF) in the first trimester placenta, but little published information about their spatiotemporal incidence.
Methods: We have used semi-thin sections and whole mount staining techniques on archival early pregnancy hysterectomy material as well as freshly-collected termination tissue.
Results: We report a description of the distribution of HAF, their gestational profile, and some characteristics of the constituent cells. We show crypt-shaped HAF are present in villi at different levels from 4 to 11 weeks and in the chorionic plate from 4 to 9 weeks. In the villous placenta, the foci often approach closely at one end to the trophoblast basement membrane. Morphologically they show remarkable similarity to those found in the yolk sac at similar stages. In some crypts, all cells are CD34+, but CD34 and nestin progressively segregate into the endothelial lineage. Brachyury is present in less differentiated cells. The erythroid lineage is dominant, as shown by the widespread expression of CD235a/glycophorin and characteristic erythroid morphologies, indicating various degrees of differentiation. However, CD41 is also present in non-endothelial cells. Initially a discontinuous UEA-1/CD31-positive endothelium forms at the periphery of the foci. These cells appear to become integrated into the developing vasculogenic/angiogenic vessel network. We also demonstrate that, independent of HAF, vasculogenesis occurs near the tips of growing villi during the first trimester.
Discussion: We suggest HAF interface with the developing vascular network, producing communication channels that allow erythrocytes to enter the placental-embryonic circulation. We speculate that the erythroid cells act as oxygen reservoirs during the period before flow of maternal blood through the intervillous space of the placenta, allowing a slow feed of oxygen-rich cells to the developing embryo.

1. Introduction

In the second week of gestation, the outer trophoblast layers of the implanted human embryo are invested by radial tongues of outgrowing extraembryonic mesenchyme. From day 14 post conception, vascular elements begin to form within this layer, comprising cords of endothelial cells that develop into networks [1–3]. The vascularised mesenchyme forms the stroma of the chorionic plate and, through progressive outgrowth, the core of the rapidly branching and growing villous placenta. The developing placental vascular network connects though the body stalk to the gut, the developing anterior vascular plexus, the heart and yolk sac [1]. Early in the 5th week of gestation, when the heart starts beating, the placental vascular network is connected to the embryo and yolk sac via the chorionic plate and umbilical cord.

Though early studies of the placenta identified vascular elements at these stages [4], limitations in tissue preservation in the mid 20th century probably obscured hemangioblastic and/or hematopoietic activity. This was however suggested in the 1980s [5], and morphological studies as well as *in situ* cell marker evidence later emerged to support the idea that hemangioblastic sites are present in first trimester placenta [6] as well as in the yolk sac [7]. These sites comprise tightly packed cells, often with recognisable

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endothelialised margins.

More recently, colony-forming assays conducted with cells isolated from placenta have confirmed the presence of hematopoietic cells both in the first trimester and later in gestation [8]. The relative incidence of the various derivative lineages changes, with erythroid precursor cells dominating in the first 9 weeks though multilineage precursors are also present [9]. Similarly in the mouse, it is clear that in addition to the yolk sac, the placenta proper is an important hematopoietic tissue [10,11].

Late in the first trimester, villi regress over the superficial (capsular) aspect of the placenta and the chorionic plate develops into the avascular chorion laeve. Studies conducted to date have not always enjoyed access to well preserved tissue from a spectrum of early gestational ages, and generally have not addressed the question of where and when angioblastic sites are found in relation to the cord insertion. They have also not clarified the spatial and developmental relationships between developing vasculogeneic and angiogenic structures and the primitive cell populations present in putative hemangioblastic foci. Here we have combined various imaging methods, including whole mount immunofluorescence and semithin resin sections, in order to throw light on these and related questions.

2. Materials and methods

2.1. Tissue

Thirty specimens were analysed from the Manchester early pregnancy tissue bank and 18 from the Boyd collection at the Centre for Trophoblast Research (CTR) at the University of Cambridge. Pregnancy termination tissue at 4–12 weeks gestation was Carnegie-staged. Boyd tissues are staged by crown-rump length; these have been converted to an estimated gestational age to the nearest week. Two specimens were also obtained from Dr C Dunk, Toronto. Tissues were cleaned in Dulbecco's Minimal Essential Medium, then fixed in ice-cold 90% methanol overnight before rehydrating in PBS for whole mount staining. For immunoperoxidase staining, tissue was fixed in neutral buffered formalin overnight then washed and processed into paraffin wax. Efforts were made to minimise the time between collection and fixation. A period of <4 h gave acceptable histological preservation.

Whole mount immunofluorescence was carried out essentially as described [12] using directly conjugated monoclonal antibodies to CD235a (Alexa 488 conjugate, BD Pharmingen 559943, 1/200), CD41 (Alexa 488 conjugate, MEM-06, AbCam, 1/50) or CD31 (Alexa 647 conjugate, WM59, BioLegend, San Diego, CA, 1/40).

2.2. Immunohistochemistry

Sections (6 μm) were heated at 60 °C for 20 min to soften the wax then deparaffinised in Histoclear (3 × 5 min) and rehydrated in alcohol (100% ethanol 2 × 3 min, 70% alcohol 3 min) then water. They were microwaved (10 min) in citrate buffer pH 6.0 (0.01 M), cooled for 20 min then endogenous peroxidise activity was blocked using 400 ml of methanol containing 1.6 ml 1 M HCl/litre with 2 ml H_2O_2. After rinsing in running water and TBS a 5% bovine serum albumin protein block was applied. The sections were incubated overnight at 4 °C with primary antibodies: monoclonal mouse anti-CD34 (Dako, QBend10, 0.18 μg/ml), monoclonal mouse anti-nestin (10 μg/ml), monoclonal mouse anti-CD45 (Dako, 2B11 + PD7/26, 7 μg/ml), monoclonal mouse anti-CD68 (Dako, PG-M1, 0.4 μg/ml), monoclonal rabbit antibody Flk-1 (AbCam, 10C2, 2.5 μg/ml), polyclonal goat anti-brachyury (Santa Cruz, C-19, 4 μg/ml, 2 μg/ml, 1 μg/ml). Controls: mouse IgG (10 μg/ml) for nestin and CD34, mouse IgG (7 μm/ml) for CD41, CD45 and CD68, rabbit IgG (2.5 μg/ml) for FLk-1 and TBS (0.125 M) for brachyury. Sections were incubated with secondary antibody: polyclonal goat anti-mouse, polyclonal swine anti-rabbit or polyclonal rabbit anti-goat followed by avidin peroxidise (5 μg/ml in 0.125 TBS). The tissue sections were washed with TBS (0.125 M) and peroxidase activity was visualised by application of diaminobenzidine, then counterstained with hematoxylin. Finally the tissue was dehydrated in alcohol (70% 3 min, 100% 2 × 3 min) and then cleared in histoclear and mounted in DPX.

2.3. Semithin sections and lectin histochemistry

Specimens of placenta of 4, 6, 7 and 8 weeks' gestation and a 6 week yolk sac were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer pH 7.3 for 4 h, then washed in buffer containing 3 mM calcium chloride several times over 24 h before being dehydrated and embedded in TAAB epoxy resin (TAAB Laboratory Equipment Ltd., Aldermaston, UK). Sections 0.5 μm thick were cut and stained with 1% toluidine blue in 1% borax for 2 min on a hotplate at 60 °C then rinsed in water. Suitable areas were selected for lectin histochemistry and 0.75 μm thick sections were mounted on 3-aminopropyltriethoxysilane-coated slides and dried at 50 °C for 48 h after which they were stained with a panel of 5 lectins as previously described [13]. These were agglutinins from *Ulex europaeus-1, Solanum tuberosum, Phytolacca americana, Maackia amurensis* (all from Vector Laboratories Ltd, Peterborough, UK) and *Sambucus nigra-1* (E-Y Laboratories, San Mateo, CA, USA) which bind to fucose (UEA-1), N-acetyl glucosamine oligomers (STA, PAA), α2,3 sialic acid (MAA)and α2,6-sialic acid (SNA-1). These lectins were selected from a panel of 25 as a previous study (Jones et al., 2015) had indicated that they show binding to cells of the erythroid lineage. Briefly, after resin removal with 50% sodium ethoxide, blocking endogenous peroxidase and subjecting the sections to a brief trypsinization step, sections were stained with 10 μg/ml (UEA-1, STA, PAA, MAA) or 50 μg/ml (SNA-1) biotinylated lectin in 0.05 M TBS pH 7.6 with 1 mM added calcium chloride for 1 h at 37 °C then treated with 5 μg/ml avidin peroxidase (Sigma) in 0.125 M TBS, pH 7.6, with 0.347 M sodium chloride for 1 h at 37 °C [14]. Sites of lectin binding were revealed with 0.05% diaminobenzidine tetrahydrochloride dihydrate (Sigma) in 0.05 M TBS, pH 7.6, and 0.015% hydrogen peroxide (100 volumes) for 5 min at room temperature. Sections were rinsed then lightly counterstained with Harris's hematoxylin before mounting in DPX. Images were captured on an Olympus microscope using Image-ProPlus software (Media Cybernetics, Rockville, MD, USA).

3. Results

3.1. Morphology and glycosylation of HAF

Flat-mounted unfixed specimens of first trimester villous placenta from the end of the first month (Fig. 1) showed elongated hemoglobin-rich structures, often with bulbous tips approaching close to, or in direct contact with, the trophoblast basement membrane, though not in all villi. They appeared to connect to channels lacking visible hemoglobin that continued along the axis of the villi.

Histological wax and semi-thin resin sections of tissue fixed soon after delivery revealed prominent cleft-shaped or ellipsoid structures in the villous mesenchyme alongside small open vessel profiles (Figs. 2 and 3), sometimes called 'blood islands', here referred to as hemangioblastic foci (HAF), interacting closely with basal cytotrophoblast (Fig. 3). In some sections the clefts were seen to comprise branching networks in villous mesenchyme (Fig. 2A). Simple open endothelialised vascular channels were also seen,