Expression of fibrillar proteins and vimentin in developing chorionic villi is related to fetal maturation

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Summary

Using an immunocytochemical technique, the extracellular matrix components fibronectin, vimentin, laminin and collagen type IV were investigated in human chorionic villi of various stages of development. Fibronectin and laminin were consistently positive throughout embryonic development. Vimentin and collagen type IV were negative in first and second trimester chorionic villi, but became positive in term placentas. With the exception of laminin, all extracellular matrix molecules were detected in the villous stroma and, with the exception of vimentin, they were localized in the basement membranes. Our data suggest that fibronectin and laminin are essential components of the villous structure, while the presence of vimentin and collagen type IV in the chorionic villi should be regarded as an indicator of fetal maturation.

Key words: Fibrillar proteins; Vimentin; Chorionic villi; Fetal maturation; Laminin; Collagen type IV; Fibronectin.

Introduction

Human chorionic villi display the same structure throughout gestation. They are composed of an outer syncytiotrophoblastic layer and an inner cytotrophoblastic layer which surround a central mesenchymal stromal core containing primitive connective tissue cells, connective tissue fibres and blood vessels. Deep in the trophoblast (the syncytiotrophoblastic and the cytotrophoblastic layer taken together) lies a basement membrane which surrounds the mesenchymal villous stroma.

As has been shown by immunocytochemical techniques, the essential matrix components of the villous stroma contain several types of fibrillar proteins (collagen types I, III, IV, V and VI, fibronectin) [1-5], glycosaminoglycans (heparan sulfate) [6] and extracellular structural glycoproteins (tenascin and laminin) [7-9].

However, the expression of these molecules during the various stages of placental development has received little attention. Therefore, the purpose of this study was to investigate the distribution of the essential extracellular matrix components in the first, second and third trimester chorionic villi.

Materials and Methods

Fourteen specimens of chorionic villi in various stages of embryonic development were retrieved from the files of the Department of Pathology, Democritus University of Thrace, Alexandroupolis, Greece. The tissues had been fixed in 10% formol saline and processed routinely in paraffin wax.

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Immunoperoxidase studies were performed using the universal streptavidin biotin detection system (Kwik Kit, Shandon Lipshaw, Pittsburgh, PA, USA). The primary antibodies used were fibronectin, vimentin, collagen type IV and laminin.

In this procedure, 5 m sections were deparaffinized and rehydrated, then treated with 3% hydrogen peroxide in methanol for 30 min in the dark at room temperature to remove endogenous peroxidase activity. Sections to be tested using antibodies requiring trypsin digestion (fibronectin) were incubated at 37°C for 20 minutes in 0.25% trypsin (Lipshaw Immunon Pittsburgh, Cat No. 484435). All sections were then incubated with PBA (protein blocking agent) for 10 minutes to reduce nonspecific background staining, primary antibody for 20 minutes, biotinylated anti-rabbit and anti-mouse immunoglobulins (linking antibody) for 10 minutes, and streptavidin peroxidase reagent (labeling antibody) for 10 minutes. These incubations were prepared at 37°C; between incubations, except for the blocking serum, sections were washed with cadenza buffer (Shandon Cat No. 47340). For colour development chromogen diaminobenzidine (DAB) (Lipshaw Immunon Pittsburgh) with 0.01% hydrogen peroxide was used, followed by counterstaining with Harris' haematoxylin and mounting with Entellan (Merck).

Positive staining was identified by the presence of a brown reaction product. Suitable positive and negative controls were included.

Results

The results are shown in Table 1. Fibronectin and laminin were consistently expressed throughout placental development. Fibronectin immunoreactivity was initially (7-10 weeks' gestation) weak and was detected in the villous stroma and the fetal basement membranes (Figure 1). Subsequently (second and third trimester chorionic

Table 1. — *The immunocytochemical reaction of chorionic villi in different stages of development.*

Chorionic villi	Fibronectin	Vimentin	Collagen Type IV	Laminin
First trimester	(+)	(–)	(–)	(+)
Second trimester	(+)	(–)	(–)	(+)
Third trimester	(+)	(+)	(+)	(+)

villi), the intensity of the reaction considerably increased, particularly at term, although the distribution of the stain remained unaltered. Laminin reactivity was restricted to the trophoblastic basement membranes and the fetal capillary walls (Figure 2).

Vimentin and collagen type IV, on the other hand, were unreactive in the first and second trimester placentas, but were positive at term. Vimentin was confined to the villous stroma, while collagen type IV reacted with both basement membranes and the villous stroma.

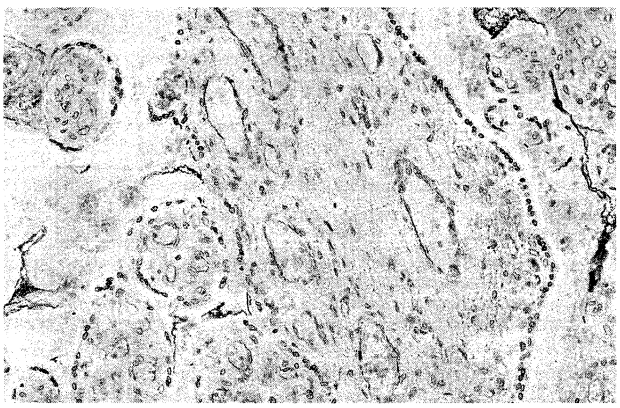

Figure 1. — Localization of fibronectin in the villous stroma and the fetal blood vessel walls x200 (Immunoperoxidase technique).

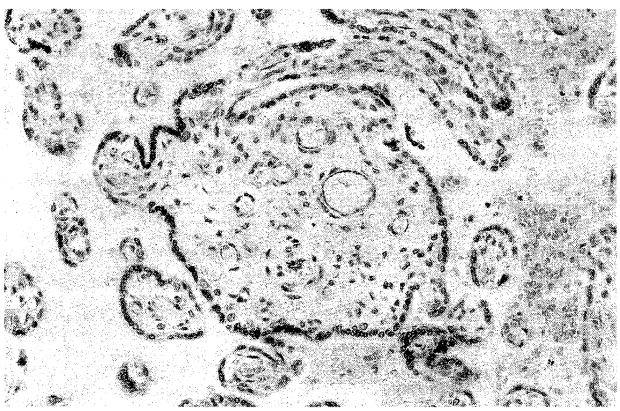

Figure 2. — Laminin reactivity restricted to the fetal basement membranes x100 (Immunoperoxidase technique).

Discussion

This study indicates that laminin is an essential component of the trophoblastic and fetal capillary basement membranes, while fibronectin is an indispensable constituent, particularly of the early human placenta [9]. Laminin is involved in various interactions with other basement membranes [10]. Fibronectin is equally important since it appears to have a role in directing and organizing cells to form a villous structure [1]. Comparable results have been reported by others [6, 9].

Interestingly, not only vimentin but also collagen type IV is detected in the villous stroma. This observation has been reported previously by Nanaev [12] in developing human placenta. Collagen type IV, like laminin, is found in the trophoblastic and fetal capillary basement membranes, but unlike laminin, it is only present during the third trimester, being unreactive in the preceding period. The same applies to the mesenchymal component vimentin. These obsevations suggest that the expression of vimentin and collagen type IV in the chorionic villi play an important role in the maturation of chorionic villi and may be regarded as markers of fetal differentiation.

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