Distribution of Fibronectin and Other Connective Tissue Components in Human Placenta

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Department of Biochemistry, *Department of Internal Medicine, and †Department of Pathology, Tohoku University School of Medicine, and ‡Department of Medicine, Research Institute for Tuberculosis and Cancer, Tohoku University, Sendai 980

Isemura, M., Yamaguchi, Y., Munakata, H., Kurosawa, K., Furuyama, T., Yoshinaga, K., Masuda, T., Nagai, H., Motomiya, M. and Yosizawa, Z. Distribution of Fibronectin and Other Connective Tissue Components in Human Placenta. Tohoku J. exp. Med., 1985, 145 (4), 373-379 — Human placenta specimens obtained at term were investigated for distribution of fibronectin, collagens and glycosaminoglycans. When examined by the immunofluorescence staining technique with anti-plasma fibronectin antiserum, fibronectin was shown to be present around the fetal blood vessels and in the stroma of placental villi. The distribution of type IV collagen also was examined with specific antiserum. It was found that its distribution was similar to that of fibronectin. Conventional alcian blue staining indicated that the placental villi contained only small amounts of glycosaminoglycans. These data suggest that fibronectin and type IV collagen play important roles in tissue organization of the placental villi. —— fibronectin; collagen; glycosaminoglycan; placenta; immunofluorescence

Fibronectin is a high molecular weight glycoprotein found in blood plasma, in connective tissues, and on the surface of a number of cell types (Ruoslanti et al. 1981; Yamada 1982, 1983). Tissue fibronectins have been detected by immunofluorescence technique (Kojima et al. 1981; Ruoslanti et al. 1981; Yamada 1982). However, the distribution of this glycoprotein in the human placenta has not been reported. In our previous work, we have isolated and characterized the fibronectin associated with placental tissues (Isemura et al. 1984b).

In the present paper, the distribution of fibronectin together with that of collagens and glycosaminoglycans is described.

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MATERIALS AND METHODS

Placenta specimen. Placentas were obtained at term from pregnant women without complications. The tissues were processed for immunofluorescence staining as described previously (Kojima et al. 1981).

Preparation of placenta fibronectin. Fibronectin was extracted and isolated from the placenta as described previously (Isemura et al. 1984b).

Collagen preparations. Various types of collagen were prepared by pepsin digestion of human fetal membranes, followed by selective precipitation with NaCl according to the method described by Burgeson et al. (1976). Calf skin type I collagen (C 3511, Type III) and human placenta type IV collagen (C 7521, Type VI) were obtained from Sigma Chemical Co. (St. Louis, U.S.A.), and the latter was used for the preparation of anti-type IV collagen antibody.

Antiserum. Antiserum against human plasma fibronectin was prepared as described previously (Kojima et al. 1981). Anti-type IV collagen antiserum was prepared by immunizing rabbits with type IV collagen (Sigma Type VI) by the method similar to that described for the preparation of anti-fibronectin antiserum (Kojima et al. 1981).

Immunohistochemical technique. Immunofluorescence staining with specific antiserum and fluorescein isothiocyanate-conjugated goat anti-rabbit gamma-globulin (Cappel Laboratories Inc. U.S.A.) was performed as described previously (Kojima et al. 1981). In control experiments, the antiserum was substituted either with pre-immune rabbit serum or with phosphate-buffered saline.

Histochemistry. Tissue sections fixed in formalin were stained with hematoxylin-eosin, with Azan-Mallory, and with alcian blue at pH 4.0 respectively.

Immunochromic technique. Enzyme-linked immunosorbent assay was carried out according to the method described previously (Isemura et al. 1984a). Ouchterlony double immunodiffusion was performed as described previously (Isemura et al. 1981).

RESULTS

Specificity of antiserum

Placenta fibronectin formed a precipitin line with monospecific anti-plasma fibronectin antiserum in an Ouchterlony double immunodiffusion system (Fig. 1). Complete fusion of the precipitin line of the placental fibronectin with that formed between plasma fibronectin and the anti-plasma fibronectin antiserum

Fig. 1. Immunological cross-reactivity between placenta fibronectin and human plasma fibronectin. Ouchterlony double immunodiffusion was performed in 1% agarose gel as described previously (Isemura et al. 1981).

Fig. 2. Specificity of rabbit anti-type IV collagen antibody. Plastic microtiter plates coated with a serial 3-fold dilution of various types of collagen were incubated with 100-fold dilution of the antiserum against human placenta type IV collagen. The plates were washed and then incubated with 1000-fold dilution of peroxidase-conjugated anti-rabbit immunoglobulin G. The peroxidase activity associated with the plates was measured at 492 nm as described previously (Isemura et al. 1984a). Collagens used for coating were calf skin type I (●), human placenta type III (▼), type IV (○), and type V (□).

Fig. 3. Immunofluorescence staining for fibronectin in the human term placenta section. Left, Fibronectin was detected around the fetal blood vessels and in the stroma of the villi. Fibronectin was virtually absent in the trophoblasts and along the trophoblastic basement membranes. Right, Control experiment with pre-immune rabbit serum.
indicated that fibronectins from plasma and placenta are immunochemically indistinguishable.

Monospecificity of anti-type IV collagen antiserum was confirmed by the solid-phase enzyme immunoassay (Fig. 2).

**Distribution of fibronectin**

Fibronectin was detected in abundance around the fetal blood vessels and, to a lesser extent, in the stroma of term placental villi, but not in the trophoblasts.
of the villous epithelium (Fig. 3). The trophoblastic basement membranes were not positive for fibronectin in most of the specimens examined (Fig. 3), although in a few specimens they were stained weakly.

**Distribution of collagens**

Azan-Mallory staining of the tissue sections indicated that collagens were present in the stroma of the villi (Fig. 4). The trophoblastic basement membranes were also positive for collagens (Fig. 4). Immunohistologic technique with monospecific antiserum revealed that type IV collagen was present around the fetal blood vessels (Fig. 5). The basement membranes were positive for type IV collagen (Fig. 5).

**Distribution of glycosaminoglycans**

The placental villi were only faintly stained with alcian blue (Fig. 6).

**Discussion**

Fibronectin was mainly detected around the fetal blood vessels and in the stroma of placental villi. Fibronectin extracted from the human placenta has been shown to be different from plasma fibronectin in apparent molecular weight and carbohydrate structure (Isemura et al. 1984b; Zhu et al. 1984). However, they are very similar to each other with respect to amino acid compositions, cell attachment-promoting activities and immunochemical properties (Isemura et al. 1984b). Therefore, no placenta-associated fibronectin is considered to be of plasma origin. Its synthesis by resident cells in the tissue is more likely. The
cells which are possibly responsible for its production would be the endothelial cells of fetal blood vessels and fibroblasts of the stroma in view of their known ability in vitro to synthesize this glycoprotein (Ruoslhti 1981; Yamada 1982). Future work is needed to prove this possibility.

Distribution of type IV collagen in the stroma and the fetal blood vessels was very similar to that of fibronectin. Apparently these data are in accord with the previous findings which showed the co-distribution of fibronectin and type IV collagen in fibrotic liver diseases (Hahn et al. 1980), and thus suggest their close association in the tissues.

The trophoblastic basement membranes, however, showed a significant difference as to the distribution of fibronectin and type IV collagen in that the former was virtually absent. These findings suggest that the organization of trophoblastic basement membranes is independent of other connective tissue structures in chorionic villi of the term placenta.

The placental villi were shown to contain only small amounts of glycosaminoglycans as revealed by alcian blue staining. Thus, the present data indicate that fibronectin and type IV collagen are the most abundant connective tissue components and that they play important roles in the tissue organization of placental villi. However, the possibility of other types of collagen (which were not included in the present study) participating therein can not be excluded.

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References

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