Increase in the relative level of type V collagen during development and ageing of the placenta

M Iwahashi, A Ooshima, R Nakano

Abstract

**Aim**—To obtain some insight into the extracellular matrix in the placenta, changes in the composition of collagens during placental development were investigated.

**Methods**—Collagen was extracted from placentas (group 1, 25–30 weeks, n = 21; group 2, 31–36 weeks, n = 32; and group 3, 37–41 weeks of gestation, n = 40) and the relative concentrations of various collagens were evaluated by SDS-PAGE.

**Results**—The ratio of the intensity of the α1(III) band to that of α1(I) chain collagen in group 3 placentas were lower than those in group 1 placentas. In contrast, the ratio of the intensity of the α1(V) band to that of α1(I) chain collagen in group 3 placentas were higher than those in group 1 and group 2 placentas.

**Conclusions**—These results suggest that type V collagen might play an important role in the function of the placenta and that an increased relative concentration of type V collagen might be closely associated with the development and ageing of the placenta.


Keywords: type V collagen, placenta, ageing.

As the placenta grows and ages, certain types of histological change occur. Such changes include a decrease in the thickness of the syncytiotrophoblast, a decrease in the stroma, thickening of the basement membrane of capillaries and trophoblasts, obliteration of certain vessels, and deposition of fibrin on the surface of the villi.9

The extracellular matrix (ECM) is considered to play an important role in the stability of tissue structure and in the regulation of cell growth and differentiation.23 The distribution of components of the ECM, such as various collagens, fibronectin, and laminin, in the placenta has been studied by immunohistochemical methods.9,10 However, little is known about the change in the composition of the ECM in the placenta during development and ageing. Among the various collagens, type V collagen was originally described as a component of chorionic and amniotic membranes.7 It is thought to play a major role in maintaining a barrier against pathogens and inflammatory cells, and in preventing the loss of amniotic fluid.8 In the present study, we attempted to investigate relative concentrations of type V collagen in extracts of placental tissue in the second and third trimester of pregnancy.

**Methods**

This study was approved by the Committee on Investigations Involving Human Subjects, Wakayama Medical College. Informed consent was obtained from each subject after the purpose and nature of the study had been explained fully.

**TISSUE**

Ninety three placentas (group 1, 25–30 weeks, n = 21; group 2, 31–36 weeks, n = 32; and group 3, 37–41 weeks of gestation, n = 40), obtained at vaginal delivery from women aged 19–39 years with uncomplicated pregnancies, were investigated. The chorion and amnion were removed, and specimens were cut from three separate central zones. Necrotic, infarcted and haemorrhagic areas were excluded.

**SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) OF PEPSIN SOLUBILISED COLLAGENS**

Minced samples of placenta were washed overnight in cold distilled water to remove any blood. Tissues were homogenised with a Polytron homogenizer in 50 volumes of 0.5 M acetic acid containing 1 mg/ml pepsin (Sigma, St Louis, Missouri, USA). Collagens were extracted with constant stirring for 24 hours at 4°C. The solutions were centrifuged at 39 000 × g for one hour at 4°C. Collagens were re-extracted from the pellets under the same conditions over 48 hours. The respective supernatants were then combined and collagens were precipitated by addition of 4.0 M NaCl to a final concentration of 2.0 M. Each precipitate was dissolved in 0.5 M acetic acid and the solution was dialysed against 0.02 M Na₂HPO₄. Precipitated collagens were redisolved in 0.5 M acetic acid, dialysed exhaustively against 0.05 M acetic acid and finally lyophilised. The solubility of the tissue collagen from each placental sample was estimated by comparing the hydroxyproline content of the initial homogenate with that of the final solution of collagen.6 Type V collagen was isolated by salt precipitation from pepsin digests of placental tissues, as described elsewhere.10,11
The extracted type V collagen was also lyophilised. Estimations of the relative abundance of the α1(III) and α1(V) chains were made by interrupted gel electrophoresis, as described by Sykes et al.10 Electrophoresis was performed in an 8% polyacrylamide gel slab (Sigma); 0.1 M phosphate buffer, pH 7.2, containing 0.1% SDS (Nacalai Tesque, Kyoto, Japan), was used to bathe the gel and electrode, as described by Laemmli.11 Lyophilised samples of placental collagens and type V collagen were dissolved at a concentration of 0.2 mg/ml and denatured by heating in the gel buffer containing 1% SDS at 60°C for 30 minutes. Aliquots of 25 ml solutions of denatured collagens and 5 ml denatured type V collagen were loaded onto the gel and subjected to electrophoresis at 80 mA. After 90 minutes the current was switched off and sample wells were filled with a 20% solution of β-mercaptoethanol (Wako, Osaka, Japan) in gel buffer, which was allowed to diffuse into the gel for one hour to cleave the intramolecular disulphide bonds of type III collagen [α1(III)]. Electrophoresis was then resumed and allowed to continue for another hour. Each collagen α chain was stained with Coomassie brilliant blue (Sigma) and intensities of bands were quantitated by densitometry. The relative amounts of α1(III) or α1(V) chains were calculated by dividing the intensities of band areas under densitometric peaks of α1(III) and α1(V) by that of α1(I).

Statistical Analysis
The ratios of α1(III) to α1(I) chains and of α1(V) to α1(I) chains, as estimated by densitometry, are expressed as mean (SEM). Results were analysed by analysis of variance and unpaired t tests.

Results
Although the relative concentrations of α1(I) were similar in the three groups, those of α1(V) increased and those of α1(III) decreased during placental development and ageing from the second to the third trimester of pregnancy (fig 1). The ratios of intensities of bands of α1(III) to α1(I) (fig 2A) were 0.95 (0.17), 0.68 (0.21) and 0.41 (0.14); and those of α1(V) to α1(I) (fig 2B) were 0.12 (0.01), 0.18 (0.04) and 0.30 (0.06) for groups 1, 2 and 3, respectively. The mean ratio of the intensity of the α1(III) band to that of α1(I) in group 3 placentas was significantly lower than that in group 1 placentas (p < 0.05). By contrast, the mean ratio of the intensity of the α1(V) band to that of α1(I) in group 3 placenta was significantly higher than in group 1 (p < 0.01) and group 2 (p < 0.05) placentas.

Discussion
In the present study, we investigated changes in the composition of collagens in placentas during the second and third trimesters of pregnancy. We were able to solubilise 70–85% of collagen in the human placental tissues, as measured by reference to hydroxyproline.
interactions of this collagen with thrombospordin and heparan sulphate might be important in the assembly of the ECM and in the regulation of its biological functions. Therefore, it is suggested that increased relative concentrations of α1(V) chains or type V collagen in the placenta might provide a biochemical basis for the functional role of the placental ECM in the synthesis of type III V collagen.

In conclusion, the placenta at term seems to be characterised by increased relative concentrations of type I and type V collagens. Our results suggest that alterations in the composition of collagen during placental development and ageing might play important roles in the invasion of trophoblastic cells, the supply of nutrients to the developing foetus and in maintaining pregnancy.

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