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

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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.**

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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 syncytium, partial disappearance of Langerhans cells, 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.[1]

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.[2,3] The distribution of components of the ECM, such as various collagens, fibronectin, and laminin, in the placenta has been studied by immunohistochemical methods.[4–6] 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 Na_2HPO_4. Precipitated collagens were redissolved 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.[9] Type V collagen was isolated by salt precipitation from pepsin digests of placental tissues, as described elsewhere.[10,11]

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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.*[12] 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.[13] 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 $[\alpha1(III)]_3$. 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 placentas was significantly higher than that in group 1 ($p < 0.01$) and group 2 ($p < 0.05$) placentas.

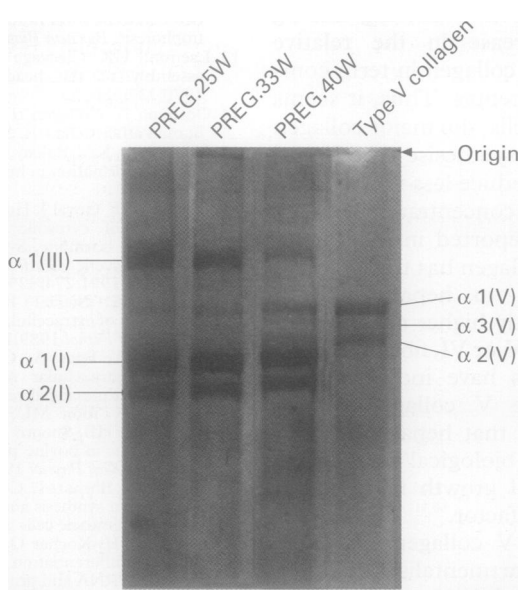

Figure 1 SDS-PAGE of pepsin solubilised collagens. Lane 1, 25 week old placenta; lane 2, 33 week old placenta; lane 3, 40 week old placenta; lane 4, purified type V collagen.

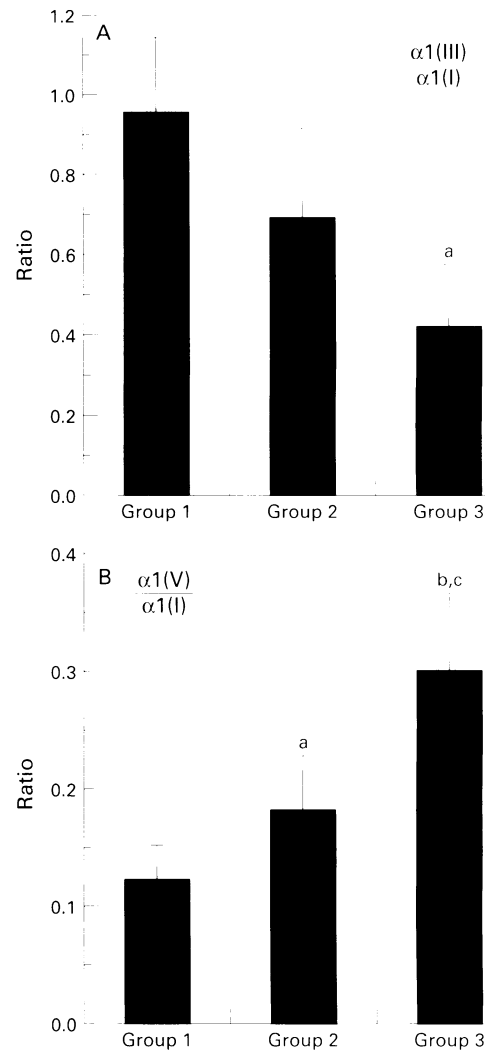

Figure 2 Relative abundance of the (A) α1(III) and (B) α1(V) collagen chains compared with the α1(I) chain in group 1 (n = 36), group 2 (n = 42), and group 3 placentas (n = 53). Data are expressed as mean (SEM). Values were analysed for statistical significance of differences among the three groups by analysis of variance and unpaired t tests: [a]p < 0.05 versus group 1 placentas; [b]p < 0.01 versus group 1 placentas; [c]p < 0.05 versus group 2 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

concentrations (data not shown). Therefore, we postulated that the extracted collagen might accurately reflect the entire complement of collagen in the sample tissues.

Although type I and type III collagens are commonly found in combination, the ratios of type III to type I collagen in group 1 placentas were significantly higher than those in group 3 placentas. Changes in the ratio of type III to type I collagen have been demonstrated in skin on ageing[12] and during the development of atherosclerosis.[14 15] A possible cause of changes in the composition of the ECM in the placenta might be an alteration in the hormonal environment. Another possible cause might be an alteration in the density of cells in the placenta. Recently, cell density dependent effects have been reported in the various cell types, such as mesangial cells,[16-18] endothelial cells,[19] vascular smooth muscle cells,[20-22] fibroblasts,[23 24] and primitive mesenchymal cells.[25] It has been suggested that cell density might modulate biological behaviour, with changes in signal transduction responses to hormonal stimulation, in growth, in the synthesis and composition of the ECM, and in the synthesis of specific proteins.[16-18] Wolthuis *et al*[18] reported that mesangial cells synthesised relatively more type I collagen per cell at higher cell densities, whereas rates of synthesis of type III and type IV collagens in each cell did not depend on cell density.

The distribution of type V collagen in the placenta has been determined by immunohistochemical staining.[5] Changes in the relative concentration of this collagen during placental development have not been clarified fully. In the case of type V collagen, the purified protein extracted from placentas by differential salt precipitation was composed of α1(V), α2(V) and α3(V) chains, with α1(V) chains predominating. This finding is consistent with previously reported data.[26] Therefore, the relative concentrations of α1(V) were calculated in terms of α1(I). A notable increase in the intensity of the α1(V) band was very evident in group 3 placentas. The most striking finding was a significant increase in the relative concentration of type V collagen in term compared with preterm placentas. Thus, it seems that placental stromal cells, the major collagen producing cells, mainly synthesise type V and type I collagens, and produce less type III collagen. Increased relative concentrations of type V collagen have been reported in atherosclerotic tissue.[14] Type V collagen has the ability to bind to insulin[27] and to heparin/heparan sulphate[28] with apparently higher affinity than collagen types I, II, III, IV, or VI, fibronectin, or laminin. Recent studies have indicated that insulin bound to type V collagen retains mitogenic activity[27] and that heparin/heparan sulphate modulates the biological activities of vascular endothelial cell growth factor[29] and basic fibroblast growth factor.[30 31] These findings suggest that type V collagen might be important in the compartmentalisation, storage, stabilisation, and modulation of the activities of various growth factors. As type V collagen also binds to thrombospondin,[32] the interactions of this collagen with thrombospondin 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 placenta in the development of the foetus.

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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