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 $\alpha 1(\text{III})$ band to that of $\alpha 1(\text{I})$ chain collagen in group 3 placentas were lower than those in group 1 placentas. In contrast, the ratio of the intensity of the $\alpha 1(\text{V})$ band to that of $\alpha 1(\text{I})$ 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.

(*J Clin Pathol* 1996;49:916-919)

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 $39\,000 \times g$ 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}

Department of
Obstetrics and
Gynaecology,
Wakayama Medical
College, Wakayama,
Japan

M Iwahashi
R Nakano

Department of
Pathology
A Ooshima

Correspondence: Dr R
Nakano, Department of
Obstetrics and Gynaecology,
Wakayama Medical College,
27 Shichibancho,
Wakayama 640, Japan.

Accepted for publication
12 August 1996

The extracted type V collagen was also lyophilised. Estimations of the relative abundance of the $\alpha 1(\text{III})$ and $\alpha 1(\text{V})$ 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 [$\alpha 1(\text{III})$]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 $\alpha 1(\text{III})$ or $\alpha 1(\text{V})$ chains were calculated by dividing the intensities of band areas under densitometric peaks of $\alpha 1(\text{III})$ and $\alpha 1(\text{V})$ by that of $\alpha 1(\text{I})$.

STATISTICAL ANALYSIS
The ratios of $\alpha 1(\text{III})$ to $\alpha 1(\text{I})$ chains and of $\alpha 1(\text{V})$ to $\alpha 1(\text{I})$ 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 $\alpha 1(\text{I})$ were similar in the three groups, those of $\alpha 1(\text{V})$

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) $\alpha 1(\text{III})$ and (B) $\alpha 1(\text{V})$ collagen chains compared with the $\alpha 1(\text{I})$ 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: **p* < 0.05 versus group 1 placentas; **p* < 0.01 versus group 1 placentas; **p* < 0.05 versus group 2 placentas.

increased and those of $\alpha 1(\text{III})$ decreased during placental development and ageing from the second to the third trimester of pregnancy (fig 1). The ratios of intensities of bands of $\alpha 1(\text{III})$ to $\alpha 1(\text{I})$ (fig 2A) were 0.95 (0.17), 0.68 (0.21) and 0.41 (0.14); and those of $\alpha 1(\text{V})$ to $\alpha 1(\text{I})$ (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 $\alpha 1(\text{III})$ band to that of $\alpha 1(\text{I})$ 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 $\alpha 1(\text{V})$ band to that of $\alpha 1(\text{I})$ in group 3 placentas was significantly higher than that 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

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 ageing¹² 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 $\alpha 1(V)$, $\alpha 2(V)$ and $\alpha 3(V)$ chains, with $\alpha 1(V)$ chains predominating. This finding is consistent with previously reported data.²⁶ Therefore, the relative concentrations of $\alpha 1(V)$ were calculated in terms of $\alpha 1(I)$. A notable increase in the intensity of the $\alpha 1(V)$ 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 insulin²⁷ and to heparin/heparan sulphate²⁸ 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 activity²⁷ and that heparin/heparan sulphate modulates the biological activities of vascular endothelial cell growth factor²⁹ 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 $\alpha 1(V)$ 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.

- Cunningham FG, MacDonald PC, Gant NF, Leveno KJ, Gilstrap LC (eds). Type V collagen in the placenta. In: *Williams Obstetrics*. 19th edn. Norwalk, CT: Appleton and Lange, 1993:111-38.
- Lin CQ, Bissell MJ. Multi-faceted regulation of cell differentiation by extracellular matrix. *FASEB J* 1993;7:737-43.
- Madri JA, Basson MD. Extracellular matrix-cell interactions: dynamic modulators of cell, tissue and organism structure and function. *Lab Invest* 1992;66:519-21.
- Blankenship TN, King BF. Developmental changes in the cell columns and trophoblastic shell of the macaque placenta: an immunohistochemical study localizing type IV collagen, laminin, fibronectin and cytokeratins. *Cell Tissue Res* 1993;274:457-66.
- Nanaev AK, Milovanov AP, Domogatsky SP. Immunohistochemical localization of extracellular matrix in perivillous fibrinoid of normal human term placenta. *Histochemistry* 1993;100:341-6.
- Earl U, Estlin C, Bulmer JN. Fibronectin and laminin in the early human placenta. *Placenta* 1990;11:223-31.
- Burgeson RE, El Adri FA, Kaitila II, Hollister DW. Fetal membrane collagens: identification of two new collagen alpha chains. *Proc Natl Acad Sci USA* 1976;73:2379-83.
- Modesti A, Kalebic T, Scarpa S, Togo S, Grotendorst G, Liotta LA, *et al*. Type V collagen in human amnion is a 12-nm fibrillar component of the pericellular interstitium. *Eur J Cell Biol* 1984;35:246-55.
- Kivirikko KI, Prockop DJ. Hydroxylation of proline in synthetic polypeptides with purified procollagen hydroxylase. *J Biol Chem* 1967;242:4009-12.
- Furuto DK, Miller EJ. Isolation of a unique collagenous fraction from limited pepsin digests of human placental tissue. *J Biol Chem* 1980;255:290-5.
- Miller EJ, Rhodes RK. Preparation and characterization of the different types of collagen. *Methods Enzymol* 1982;82:33-64.
- Sykes B, Puddle B, Francis M, Smith R. The estimation of two collagens from human dermis by interrupted gel electrophoresis. *Biochem Biophys Res Commun* 1976;72:1472-80.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;227:680-5.
- Ooshima A. Collagen α B chain: increased proportion in human atherosclerosis. *Science* 1981;213:666-8.
- McCullagh KA, Balian G. Collagen characterisation and cell transformation in human atherosclerosis. *Nature* 1975;258:73-5.
- Lermioglu F, Goyal J, Hassid A. Cell density modulates the decrease of cytosolic free Ca^{2+} induced by arterial natriuretic hormone, S-nitroso-N-acetylpenicillamine and 8-bromo cyclic GMP in cultured rat mesangial cells. *Biochem J* 1991;274:323-8.
- Ishimura E, Sterzel RB, Budde K, Kashgarian M. Formation of extracellular matrix by cultured rat mesangial cells. *Am J Pathol* 1989;134:843-55.
- Worthuis A, Boes A, Grond J. Cell density modulates growth, extracellular matrix, and protein synthesis of cultured rat mesangial cells. *Am J Pathol* 1993;143:1209-19.
- Patton WF, Yoon MU, Alexander JS, Chung-Welch N, Hechtman HB, Shepro D. Expression of simple epithelial cytokinins in bovine pulmonary microvascular endothelial cells. *J Cell Physiol* 1990;143:140-9.
- Majors AK, Ehrhart L. Cell density and proliferation modulate collagen synthesis and procollagen mRNA levels in arterial smooth muscle cells. *Exp Cell Res* 1992;200:168-74.
- Campbell JH, Kocher O, Skalli O, Gabbiani G, Campbell GR. Cytodifferentiation and expression of α -smooth muscle actin mRNA and protein during primary culture of aortic smooth muscle cells. Correlation with cell density and proliferative state. *Arteriosclerosis* 1989;9:633-43.
- Goodman LV, Majack RA. Vascular smooth muscle cells express distinct transforming growth factor- β receptor

phenotypes as a function of cell density in culture. *J Biol Chem* 1989;**264**:5251-44.

23 Rösner H, Greis C, Rodemann HP. Density-dependent expression of ganglioside GM3 by human skin fibroblasts in an all-or-none fashion, as a possible modulation of cell growth in vitro. *Exp Cell Res* 1990;**190**:161-9.

24 Halme T, Vihersaari R, Penttinen R. Lysyl oxidase activity and synthesis of desmosines in cultured human aortic cells and skin fibroblasts: comparison of cell lines from control subjects and patients with Marfan syndrome or other annuloaortic ectasia. *Scand J Clin Lab Invest* 1986;**46**:31-7.

25 Tsonis PA, Goetinck PK. Cell density effects of a tumor promotor on proliferation and chondrogenesis of limb bud mesenchymal cells. *Exp Cell Res* 1990;**190**:247-53.

26 Christopher N, Fietzek PP, Van der Rest M. Human placenta type V collagens. *J Biol Chem* 1984;**259**:14170-4

27 Yaoi Y, Hashimoto K, Takahara K, Kato I. Insulin binds to type V collagen with retention of mitogenic activity. *Exp Cell Res* 1991;**194**:180-5.

28 Richard GL, Agneta H, Jeffrey DE, Steffen G, Magnus H. Binding of heparan sulfate to type V collagen. *J Biol Chem* 1989;**264**:7950-6.

29 Lobb RR, Harper JW, Fett JW. Purification of heparin-binding growth factors. *Anal Biochem* 1986;**154**:1-14.

30 Thornton SC, Mueller SN, Levine EM. Human endothelial cells: use of heparin in cloning and long-term serial cultivation. *Science* 1983;**222**:623-5.

31 Schreiber AB, Kenny J, Koalski WJ, Friesel R, Mehlman T, Maciag T. Interaction of endothelial cell growth factor with heparin: characterization by receptor and antibody recognition. *Proc Natl Acad Sci USA* 1985;**82**:6138-42.

32 Mumby SM, Rauji GJ, Bornstein P. Interactions of thrombospondins with extracellular matrix proteins: selective binding to type V collagen. *J Cell Biol* 1984;**98**:646-52.