TYPE IV COLLAGEN GENE EXPRESSION DURING MURINE PLACENTAL FORMATION DEMONSTRATED BY IN SITU HYBRIDIZATION

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The placental formation involves marked histological changes in endometrial stromal cells and extracellular matrix components in many mammalian species. It is well known that basement membrane components are increasingly produced in the process of decidualization in pregnant murine uterus\(^1\). The formation of basement membrane is important for placental development and function. We studied the localization of type IV collagen mRNA during murine placental development by means of in situ hybridization.

MATERIALS AND METHODS
Pregnant uterine horns of Wistar rats and BALB/C mice at 6, 8, 10, 12 and 14 days of gestation were used. The tissues were fixed in 4% formaldehyde in PBS at room temperature for 1 hour. Some tissues were fixed in PLP solution for cryosection for immunohistochemistry. For in situ hybridization, tissue blocks were dehydrated in a graded series of ethanol and embedded in paraffin. Transverse sections including placental tissue were cut at 5\(\mu\)m. Hybridization procedures used in this study were practically the same as those described previously\(^2\). The mouse \(\alpha_1(IV)\) collagen cDNA probe employed was a 1161-bp BamHI-XhoI fragment\(^3\). The probe was labeled with \(^3\)H-dTTP by nick translation to a specific activity of \(7\times 10^7\)cpm/\(\mu\)gDNA. Type IV collagen specific polyclonal antibody was used for avidin-biotin-complex immunostaining.

RESULTS AND DISCUSSION
The cDNA probe for mouse \(\alpha_1(IV)\) collagen hybridized to type IV collagen mRNA of parietal endodermal cells of Reichert's membrane and yolk sac endodermal cells in mice. The number of silver grains of parietal endodermal cells was more than that of yolk sac cells. Similar results were also noted in rats by using this cDNA probe. In the process of endometrial decidualization, decidual cells were immunohistochemically stained with type IV collagen antibody(Fig. A) and a moderate number of grains were noted over these cells by in situ hybridization(Fig. B). In the pregnant rat uterus on days 12 and 14, high levels of type IV collagen mRNA of fetal cytotrophoblastic cell layer
Kitaoka M et al; Type IV collagen in placenta

were noted. Both decidual cells and cytotrophoblastic cells may play a role in the barrier function of the placenta.

REFERENCES


Figure A and B. Light micrographs of rat decidualized uterine norm at 10 day gestation. Figure A. Immunohistochemical stain with rat type IV collagen antibody by ABC methods. Decidual cells(D) and basement membranes (arrow) of maternal vessels(M) in the placenta are clearly positive. Figure B. Micro-autoradiograph after in situ hybridization with $^{3}$H-labeled mouse α1(IV) collagen cDNA probe. A moderate number of grains are noted over decidual cells(D) and endothelial cells(arrow) of maternal vessels(M). Each bar indicates 20 μ.