

Distribution of versican and hyaluronan in the mouse uterus during decidualization

S. San Martin¹,
M. Soto-Suazo²
and T.M.T. Zorn¹

¹Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, SP, Brasil

²Escuela de Medicina, Facultad de Ciencias Médicas, Universidad de Santiago de Chile, Santiago, Chile

Abstract

Preparation for embryo implantation requires extensive adaptation of the uterine microenvironment. This process consists of cell proliferation and cell differentiation resulting in the transformation of endometrial fibroblasts into a new type of cell called decidual cell. In the present study, we followed the space-time distribution of versican and hyaluronan (HA) in different tissues of the uterus before and after embryo implantation. Fragments of mouse uteri obtained on the fourth, fifth, sixth and seventh days of pregnancy were fixed in Methacarn, embedded in Paraplast and cut into 5- μ m thick sections. HA was detected using a biotinylated fragment of the proteoglycan aggrecan, which binds to this glycosaminoglycan with high affinity and specificity. Versican was detected by a polyclonal antibody. Both reactions were developed by peroxidase methods. Before embryo implantation, both HA and versican were present in the endometrial stroma. However, after embryo implantation, HA disappeared from the decidual region immediately surrounding the implantation chamber, whereas versican accumulated in the same region. The differences observed in the expression of HA and versican suggest that both molecules may participate in the process of endometrial decidualization and/or embryo implantation.

Key words

- Decidualization
- Endometrium
- Proteoglycans
- Versican
- Hyaluronan
- Mouse

Correspondence

T.M.T. Zorn
Departamento de Histologia e
Embriologia, ICB, USP
Av. Lineu Prestes, 1524
05508-900 São Paulo, SP
Brasil
Fax: +55-11-3818-7309
E-mail: temtzorn@usp.br

Presented at SIMEC 2002
(International Symposium
on Extracellular Matrix),
Angra dos Reis, RJ, Brazil,
October 7-10, 2002.

Research supported by FAPESP
(Nos. 00/04078-4 and 01/12283-0).

Part of a Doctoral degree presented
by S. San Martin to the Departamento
de Histologia e Embriologia, ICB,
USP, São Paulo, SP, Brazil.

Received November 29, 2002
Accepted June 9, 2003

In mammals, the endometrial stroma acquires remarkable morphogenetic potential during pregnancy. Sensitized by the ovarian hormones estrogen and progesterone, endometrial fibroblasts undergo profound morphofunctional modifications that result in the formation of a new type of cell called decidual cell. In rodents, decidualization of the endometrium promotes a rapid and profound remodeling of many of the extracellular matrix components (1-3).

During decidualization, both glycosaminoglycans and proteoglycans are reorganized

in the uterus (4,5). Brown and Papaioannou (6) observed a loss of hyaluronan (HA) from the antimesometrial stroma. HA is a highly hydrophilic molecule that attracts large amounts of water, forming a highly hydrated gel and thereby promoting expansion of the extracellular spaces and facilitating cell migration, metastasis, and angiogenesis (7,8).

Versican is a member of a large aggregating chondroitin sulfate proteoglycan family, which consists of a core protein containing various globular domains (9). One of these domains, located near the N-terminus, spe-

cifically recognizes and binds HA with relatively high affinity (10-12). Moreover, versican may block the attachment of cells to various extracellular matrix components such as collagen I, fibronectin and laminin (13), and enhance cell proliferation (14).

In the present investigation, we studied the distribution of HA and versican during the peri-implantation period, when angiogenesis, cell migration, trophoblast invasion and cell proliferation occur.

Female Swiss mice from day four to day seven of pregnancy were used. Implantation sites were fixed for 3 h in Methacarn fixative, rinsed with absolute ethanol, and embedded in Paraplast (Oxford, St. Louis, MO, USA) at 56°C. Sections (5 µm thick) were adhered to glass slides using 0.1% poly-L-lysine (Sigma, St. Louis, MO, USA), and then dried at room temperature. Each of the succeeding steps was followed by a thorough rinse with phosphate-buffered saline (PBS).

To block endogenous peroxidase activity, the sections were treated with 3% H₂O₂ in PBS for 30 min. For versican localization, the sections were incubated for 10 min at 37°C with 0.002% trypsin (Sigma), in 0.1 M PBS, pH 7.2.

Nonspecific staining was blocked by incubating the section for 30 min with normal goat serum diluted 1:1 in PBS-10% bovine serum albumin.

HA was detected using a biotinylated fragment of the proteoglycan aggrecan, diluted 1:80 in PBS-0.3% Tween 20 for 1 h at 37°C (H. Nader, UNIFESP). Versican was detected with a rabbit polyclonal antibody (clone LF-99, L. Fisher, NIH) (15) diluted 1:100 in PBS-0.3% Tween 20 and incubated overnight at 4°C. The sections were then incubated with goat anti-rabbit biotin-conjugated IgG (Vector Laboratories, Burlingame, CA, USA) for 1 h at room temperature and diluted 1:1000 in PBS for 1 h at room temperature. All sections were incubated with the streptavidin/peroxidase complex (Vector)

for 1 h at room temperature.

Reactions were visualized using 0.03% 3,3'-diaminobenzidine in PBS with 0.03% H₂O₂. The sections were counterstained with Mayer's hematoxylin. The specificity of immunolabeling was tested by omitting the primary antibody. Semiquantitative estimations of immunoperoxidase reaction data were determined by visual observation of the intensity of the brownish precipitate in the immunoreactions. Specimens from three different animals were observed using a Nikon E600 microscope.

On day four of pregnancy, HA was present in the myometrium and endometrial stroma. The reaction was observed as thin fibrils distributed in the extracellular space. Versican was observed in the smooth muscle cells of the myometrium, in the apical region of the glandular epithelium, in the cytoplasm of cells of the endometrial stroma (mainly in the subepithelial region of the uterus), and in the region of the basement membrane of the luminal epithelium.

On day five of pregnancy, the embryo implantation induced decidualization of the endometrium by transforming endometrial fibroblasts into decidual cells. The reaction to HA was diminished around the embryo implantation crypt, but continued to be similar to the previous day in all other regions of the uterus (Figure 1A). There was an intense expression of versican in the region of the decidual cells, but it appeared only weakly in the deep stroma of the uterus, where no decidual cells exist. Versican was also present in the myometrium and in the region of the basement membrane of the luminal epithelium (Figure 1C).

On day six of pregnancy, HA was absent in the mature decidual region, although it was still present in the other regions of the uterus and was observed in Reichert's membrane, on the surface of the endoderm and in cells of the embryonic ectoderm. Versican was present in the region of the mature decidual cells and in the region of predecidual

cells. Only a weak reaction to versican persisted in the deep stroma. In the myometrium, the reaction was pronounced.

On day seven of pregnancy, HA was distributed in a similar manner to that seen on day six, although a very weak signal for it was observed among mature decidual cells (Figure 1B). On day seven of pregnancy, versican was distributed in both the decidualized and the predecidualized regions. A high concentration of versican was observed on the surface of the mature decidual cells, but not on the surface of the predecidual and nondecidualized cells (Figure 1D). Table 1 shows the distribution of HA and versican immunoreactivity in uterine tissues.

The changes in HA and versican expression observed during pregnancy indicate that both molecules may participate in the preparation of the endometrial stroma for the reception and implantation of the embryo, a process that includes remodeling of the endometrial stroma, cell proliferation and differentiation of endometrial fibroblasts into decidual cells. The presence of HA in the stroma on day four of pregnancy may facilitate the development of the edema that occurs hours before embryo implantation as a consequence of an increased permeability of blood vessels, as observed by Psychoyos (16). The presence of HA may also create a microenvironment that favors the expansion of new blood vessels during the peri-implantation period. On day five of pregnancy, the embryos have already been implanted into uterine crypts and a compact layer of decidual cells, the decida, forms the surrounding stroma. Decidualization implicates the loss of the extracellular spaces, thereby affecting the arrangement and molecular composition of the endometrial stroma (1). The disappearance of HA from the region of fully transformed decidual cells may favor the establishment of extensive extracellular junctions that characterize decidual transformation. During the same period, however, HA is maintained in the mesometrial

region, where the formation of the placenta depends on blood vessel formation. Our data indicate a close association between HA distribution in the uterus and the angiogenic process.

At the onset of decidualization, HA and versican were contrarily expressed, particularly in the region of fully transformed decidual cells. These results corroborate a pre-

Table 1. Semiquantitative evaluation of hyaluronan (HA) and versican (VER) distribution in the endometrial stroma during the peri-implantation and decidualization periods.

	Days of pregnancy										
	4		5			6			7		
	SE	DE	MD	PD	ND	MD	PD	ND	MD	PD	ND
HA	+++	+++	-	+++	+++	-	+++	+++	-	++	+++
VER	+++	+	+++	++	+	+++	+++	+	+++	+++	+

SE = subepithelial stroma; DE = deep stroma; MD = mature decida; PD = predecida; ND = nondecidualized stroma; (-) = negative reaction; (+) = weak reaction; (++) = moderate reaction; (+++) = strong reaction.

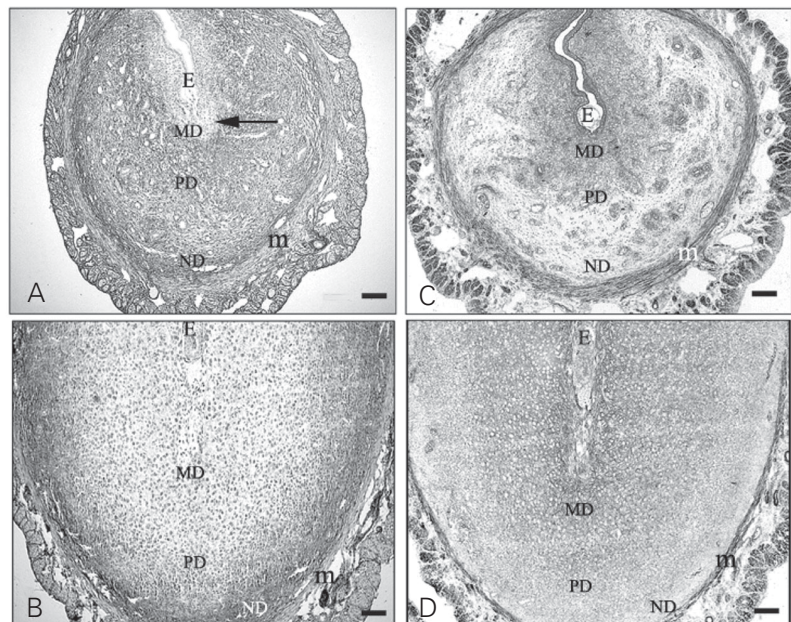


Figure 1. A, Day five of pregnancy. Hyaluronan (HA) is distributed in the predecidual and nondecidualized regions of the endometrial stroma. B, Day seven of pregnancy. HA is observed mainly in the deep stroma. C, Day five of pregnancy. Expression of versican is elevated in the decidual cell region and in the myometrium. D, Day seven of pregnancy. Versican shows a strong reaction among decidual cells and a weak reaction in the nondecidualized region. E = embryo; m = myometrium; MD = mature decida; PD = predecida; ND = nondecidualized stroma. Bar for all panels = 100 μ m.

vious report (3) showing that both the synthesis and degradation of extracellular components accompany the decidual transformation. The remodeling of the extracellular matrix appears to be necessary to provide a microenvironment in the uterus that is appropriate for receiving and developing the embryo.

This study shows, for the first time, the presence of versican in the uteri of pregnant mice before and after embryo implantation. After embryo implantation, versican was abundant in the decidualized areas formed by fully transformed decidual cells and the so-called predecidual cells, which are in the process of decidualization. However, versican was scarce in areas occupied by non-decidualized fibroblasts. Versican has been implicated in the regulation of cell migration and developing tissue pattern formation (17). A number of other reports have described the close association between chondroitin sulfate proteoglycans and barrier tissues formed to restrict neural crest cell migration and axonal outgrowth (18).

Versican belongs to the family of HA-binding proteoglycans, constituting a gene family collectively termed "hyalectins" (19). HA forms a coating around many types of cells, profoundly affecting their migratory properties and adhesiveness (20). Cell adhesion is a very important phenomenon during normal tissue development, as well as during pathogenic states such as metastasis and wound healing (21). A number of *in vitro*

observations have provided evidence for a cell adhesion destabilizing function performed by versican. For example, versican interferes with the substrate attachment of primary fibroblasts to collagen I, fibronectin, vitronectin and laminin (13). Moreover, versican present in the pericellular matrix of cultured fibroblasts is excluded from focal contacts (22). In addition to HA, versican interacts with other extracellular matrix components such as tenascin. The sustained expression of versican in decidualized regions and the disappearance of HA from the same regions exemplify the complex interrelations between the various extracellular matrix molecules in the uterus. These interactions merit future clarification. The accumulation of versican among mature decidual cells shown in the present study may be important for controlling trophoblast invasion. Moreover, the expression of versican in the regions of fully mature decidual cells suggests that versican is a genetic product of decidual transformation.

Acknowledgments

We gratefully acknowledge Dr. Alison Colqhoun for a critical reading of the manuscript. We are grateful to Drs. Larry Fisher (National Institutes of Health, Bethesda, MD, USA), Helena Nader and Lucia Sampaio (Laboratory of Molecular Biology, UNIFESP, São Paulo, SP, Brazil) for antibodies and probes.

References

1. Zorn TMT, Bevilacqua EM & Abrahamsohn PA (1986). Collagen remodeling during decidualization in the mouse. *Cell and Tissue Research*, 244: 443-448.
2. Bijovsky AT, Zorn TMT & Abrahamsohn PA (1992). Remodeling of the mouse endometrial stroma during the preimplantation period. *Acta Anatomica*, 144: 231-234.
3. Abrahamsohn PA & Zorn TMT (1993). Implantation and decidualization in rodents. *Journal of Experimental Zoology*, 266: 603-628.
4. Zorn TMT, Pinhal MAS, Nader HB, Carvalho JJ, Abrahamsohn PA & Dietrich CP (1995). Biosynthesis of glycosaminoglycans in the endometrium during the initial stages of pregnancy of the mouse. *Cellular and Molecular Biology*, 41: 97-106.
5. Greca CD, Nader HB, Dietrich CP, Abrahamsohn PA & Zorn TMT (2000). Ultrastructural cytochemical characterization of collagen-associated proteoglycans in the endometrium of mice. *Anatomical Record*, 259: 413-423.
6. Brown JJ & Papaioannou VE (1992). Distribution of hyaluronan in the mouse endometrium during the periimplantation period of pregnancy. *Differentiation*, 52: 61-68.
7. Comper W & Laurent T (1978). Physiological function of connective

- tissue polysaccharides. *Physiological Reviews*, 58: 255-315.
8. Toole B (1981). Glycosaminoglycans in morphogenesis. In: Hay E (Editor), *Cell Biology of Extracellular Matrix*. Plenum Press, New York, 259-294.
 9. Zimmermann DR & Ruoslahti E (1989). Multiple domains of the large fibroblast proteoglycan, versican. *EMBO Journal*, 8: 2975-2981.
 10. Johansson S, Hedman K, Kjellen L, Christner J, Vaheri A & Hook M (1985). Structure and interactions of proteoglycans in the extracellular matrix produced by cultured human fibroblasts. *Biochemical Journal*, 232: 161-168.
 11. Lever-Fisher PL & Goetnick PF (1988). Identification and characterization of a proteoglycan in embryonic chicken skin that can interact with hyaluronic acid. *Archives of Biochemistry and Biophysics*, 263: 45-58.
 12. LeBaron RG, Zimmermann DR & Ruoslahti E (1992). Hyaluronate binding properties of versican. *Journal of Biological Chemistry*, 267: 10003-10010.
 13. Yamagata M, Suzuki S, Akiyama SK, Yamada KM & Kimata K (1989). Regulation of cell-substrate adhesion by proteoglycans immobilized on extracellular substrate. *Journal of Biological Chemistry*, 264: 8012-8018.
 14. Zhang Y, Cao L, Yang BL & Yang BB (1998). The G3 domain of versican enhances cell proliferation via epidermal growth factor-like motifs. *Journal of Biological Chemistry*, 273: 21342-21351.
 15. Fisher LW, Stubbs 3rd JT & Young MF (1995). Antisera and cDNA probes to human and certain animal model bone matrix noncollagenous proteins. *Acta Orthopaedica Scandinavica*, 266 (Suppl): 61-65.
 16. Psychoyos A (1960). La réaction déciduale est précédée de modifications précoces de la perméabilité capillaire de l'utérus. *Comptes Rendus des Seances de la Société de Biologie et de ses Filiales*, 154: 1384-1387.
 17. Perissinotto D, Iacopetti P, Bellina I et al. (2000). Avian neural crest cell migration is diversely regulated by two major hyaluronan-binding proteoglycans. PG-M/versican and aggrecan. *Development*, 127: 2823-2842.
 18. Landolt RM, Vaughan L, Winterhalter KH & Zimmermann DR (1995). Versican is selectively expressed in embryonic tissues that act as barriers to neural crest cell migration and axon outgrowth. *Development*, 121: 2303-2312.
 19. Margolis RU & Margolis RK (1994). Aggrecan-versican-neurocan family proteoglycans. *Methods in Enzymology*, 245: 105-126.
 20. Laurent TC & Fraser JR (1986). The properties and turnover of hyaluronan. *CIBA Foundation Symposium*, 124: 9-29.
 21. Yang BL, Zhang Y, Cao L & Yang BB (1999). Cell adhesion and proliferation mediated through the G1 domain of versican. *Journal of Cellular Biochemistry*, 72: 210-220.
 22. Yamagata M, Saga S, Kato M, Bernfield M & Kimata K (1993). Selective distributions of proteoglycans and their ligands in pericellular matrix of cultured fibroblasts. Implications for their roles in cell-substratum adhesion. *Journal of Cell Science*, 106: 55-65.