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Bovine placentomal heparanase and syndecan expression is related to placental maturation

N. Hambruch, S. Kumstel, J.-D. Haeger, C. Pfarrer*

Department of Anatomy, University of Veterinary Medicine Hannover, Hannover, Germany

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ABSTRACT

Introduction: Impaired placental maturation has been associated with retention of fetal membranes, which is a major reproductive disease in cattle. This maturation includes alterations in all tissue compartments of the placenta, specifically of epithelial and stroma cells and extracellular matrix. It is believed to be controlled by hormones, adhesion molecules and proteolytic enzymes. To investigate if the proteolytic enzyme heparanase and its substrates, the syndecans (SDCs) could be involved in the release of fetal membranes, their expression in bovine placentomes was analyzed.

Methods: Placentomes were taken from gestational day 35 until term, directly after spontaneous parturition, after preterm caesarean section, and after chemically induced parturition. Heparanase and SDCs were localized by immunohistochemistry and the respective mRNAs were quantified by qRT-PCR. Heparanase expression was additionally quantified by Western blot.

Results: Heparanase, SDC1 and SDC4 displayed significant changes in expression and localization depending on gestational progress and mode of parturition. All three proteins showed an expression at the end of gestation, together with an altered, predominant localization in fetal and maternal epithelia. After physiological parturition, the placentomal tissue stained weaker for all syndecans. This change in staining pattern could not be observed after induced preterm parturition. SDC2 expression did not change during the course of gestation.

Discussion: The changing placental expression patterns of heparanase, SDC1 and SDC4 indicate that these molecules might be involved in fetomaternal communication and placental maturation in cattle. The matrix degrading properties of heparanase could assist in a timely reduction of fetomaternal adhesion and thus promote separation of the membranes after parturition.

1. Introduction

During bovine gestation the synepitheliochorial placenta establishes a tight fetomaternal connection by complementary interdigitation of fetal cotyledons with maternal caruncles. With increasing gestational age the placental exchange surface grows constantly in order to maintain the nutrition of the fetus [1]. This process requires a variety of cell-cell and cell-extracellular matrix (ECM) interactions. Towards the end of term remodeling of the ECM, for example by activation of proteolytic matrix metalloproteinases (MMPs) occurs again. However, at this time point it is discussed to be essential to ensure a successful parturition in various species [2] which includes the timely release of the afterbirth/fetal membranes.

Failure to do so results in retained fetal membranes (RFM), one of the major reproductive disorders in cattle. This disease is defined by the retention of fetal membranes in the uterus for more than 24 h postpartum, which leads to a lasting decrease of milk production and reproductive efficiency [3]. The exact cause for this symptomatology is still unknown. Among other reasons changes in cell-cell and cell-ECM interactions at the fetomaternal interface are believed to be important for the release of the fetal membranes [4]. Research regarding the causes of RFM often focusses on the most evident enzymes which are capable of degrading protein components of the ECM, like collagenases and MMPs [2,5]. Less attention was paid to enzymes cleaving other integral parts of the

* Corresponding author. Department of Anatomy, University of Veterinary Medicine Hannover, Bischofsholer Damm 15, 30173 Hannover, Germany.
E-mail address: Christiane.Pfarrer@tiho-hannver.de (C. Pfarrer).

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fetomaternal interface, namely the glycosaminoglycan chains of proteoglycans (PGs). One of those enzymes is heparanase, which has been detected in a variety of cells, including immune cells, platelets and trophoblast cells [6], and has been associated with processes like angiogenesis [7], wound healing [8], embryo implantation [9] and tissue morphogenesis [10]. The specific role of heparanase during placentation in different species is still a matter of investigation [11], yet evidence on mRNA level in the bovine placenta demonstrates that it may be important in the course of gestation [12].

The main function of heparanase is to cleave heparan sulfate (HS) chains of PGs at specific sites [13]. Beside fibrillary proteins like collagen and fibronectin, PGs are a major component of the ECM [14]. The heparan sulfate proteoglycans (HSPG) are among the most abundant cell-surface receptors and are known to be key players in many biological processes including differentiation, angiogenesis, inflammation and the regulation of basic cellular behaviour [15]. They can be divided into syndecans (SDC) and glypicans; both have one or more heparan sulfate chains (glycosaminoglycan chains) which are covalently bound to a protein core that determines the cellular localization. The complex saccharide chains provide interaction sites for different ligands like various ECM molecules [16]. Most of HSPG biological properties are directly linked to these glycosaminoglycan components since they do not only bind structural proteins, but also serve as high affinity docking sites for various signaling molecules. Growth factor binding can either potentiate their interaction with the corresponding receptor or provide localized storage depots [17]. Subsequently, rapid release of these HS-bound growth factors can be triggered by heparanase, which thereby controls cellular behaviour [13].

The four members of the syndecan family (SDC1 to SDC4) are type 1 transmembrane HSPG that interact with adhesion molecules, cytokines, coagulation factors and ECM proteins. Most cell types express at least one syndecan and these proteins are increasingly recognized as important regulators of numerous cellular processes like cell behaviour, signal transduction, immune response, and are responsible for cell adhesion [18,19]. In the human and mouse placenta they are known to be important during placental development and for ECM interactions [20,21] and they are believed to be involved in pathological processes, like preeclampsia [22,23]. While SDC3 has primarily been detected in neuronal tissue, SDC1, SDC2, and SDC4 are present in a variety of cell types, in which their expression is developmentally regulated and can be altered under certain pathophysiological conditions, including tumour onset and progression [18]. Up to date, nothing is known about the expression of syndecans in the bovine placenta in the course of gestation and at term. Due to their ability to influence growth, angiogenesis and tissue remodeling, they could be important during placentation, placental maturation and the release of fetal membranes.

Since syndecans are functional substrates of heparanase and furthermore promote the activation of this endoglycosidase [24], we analyzed the expression of SDC1, SDC2 and SDC4 by immunohistochemistry (IHC) and qRT-PCR in the bovine placenta pre- and postpartum. Additionally, this study aimed to show protein expression and localization of the corresponding cleaving-enzyme heparanase during bovine gestation and postpartum.

2. Materials and methods

2.1. Sample collection and preparation

To obtain tissue samples in the course of gestation placentomes from generally healthy, pregnant Holstein cows were collected at a slaughterhouse after routine slaughtering, while the gestational day (GD) was assessed according to fetal crown-rump length [25]. In detail, five experimental groups (three animals per group) were used (GD 30 to 80, GD 100 to 120, GD 140 to 160, GD 180 to 200, GD 250 to 270). Since the animals were killed regularly for meat production the sampling did not represent an animal experiment according to the German animal protection law. Directly after sampling, the tissue was perfusion-fixed with 4% neutral buffered formaldehyde solution (Lillie's formalin) as well as snap frozen for Western blotting.

Tissue samples from term placentae with different parturition regimes (three animals per group) were taken from a previous study [2]. All animal experiments were approved (no. V54-19c-20-15(I) Gi 18/14-Nr.41/2007; LAVES, 33.9-42502-04-09/1634) by the committee on the use of animals for research purposes at the Regierungspraesidium Giessen (Germany) and the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES) according to the German animal protection law. Briefly, placentomes were collected per vagina or caesarean section immediately after delivery of the calf from Holstein cows, which were divided into three different groups as follows (TI) preterm elective caesarean section at day 272 of gestation, (TII) chemical induced parturition at day 272 of gestation by injection of either dinoprostum (prostaglandin F2 analogue [Dinolytic/Dinoprost® 25 mg i·m.]) or dexamethasone (glucocorticoid [Dexafort® 0.06 mg/kg i·m.]) and (TIII) term parturition (spontaneous parturition) and release of fetal membranes within a period of 12 h after calving. Animals of all other groups (TI and TII) retained fetal membranes. For IHC placentome slices of 0.5 cm were fixed with Lillie's formalin for 24 h and embedded in paraffin. Human term placental tissue was used as a control for immunohistochemistry (IHC). The collection of the human tissue was approved by the ethics committee (no. 78/05) at the University of Giessen, Faculty of Medicine. Informed consent was given in all cases.

For detection of heparanase protein in bovine caruncles or cotyledons, midgestational placentomes were manually separated into the maternal and fetal parts, prior to freezing. To analyze heparanase protein expression in cell culture and to verify antibody specificity, two bovine placentomal cell lines were used: fetal trophoblast cells (F3) and caruncular epithelial cells (BCEC). The isolation, culture and characterization of the cells are described elsewhere [26,27]. For protein analysis, cells were seeded in 3.5 cm

Table 1
Sequences for the primers used.

Target	Sequence (5′ to 3′)	Amplicon	Accession No.
SDC1	For: TCACCTCATCACACACGCC Rev: GTGGACACGCTGTGAGTGG	247 bp	NM_001075924
SDC2	For: AACGGACCCAGATGAAGAGG Rev: GCACTGGATGGTTTGCGTTC	207 bp	NM_001034788
SDC4	For: GGGCAGGAATCCGATGACTT Rev: TTCCAGTTCCTTGGGTTCGG	162 bp	XM_584869
HPSE	For: GACCCCTCAAGAAGGTTTGGT Rev: GCCGACAGGCCCAATTTAT	100 bp	NM_174082
Ubiquitin	For: AGATCCAGGATAAGGAAGGCAT Rev: GCTCCACCTCCAGGGTGAT	198 bp	XM_005195028
HistonF3a	For: ACTGGCTACAAAAGCCGCTC Rev: ACTTGCCTCCTGCAAAGCAC	233 bp	XM_002684198