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Hyaluronan and its binding proteins during cervical ripening and parturition: Dynamic changes in size, distribution and temporal sequence ☆

Monika Ruscheinsky [a], Carol De la Motte [b], Mala Mahendroo [a,*]

[a] Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, TX 75390-9032, United States
[b] Department of Pathobiology, Cleveland Clinic Foundation, Cleveland, OH 44195, United States

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Abstract

The uterine cervix undergoes changes during pregnancy and labor that transform it from a closed, rigid, collagen dense structure to one that is distensible, has a disorganized collagen matrix, and dilates sufficiently to allow birth. To protect the reproductive tract from exposure to the external environment, the cervix must be rapidly altered to a closed, undistensible structure after birth. Preparturition remodeling is characterized by increased synthesis of hyaluronan, decreased expression of collagen assembly genes and increased distribution of inflammatory cells into the cervical matrix. Postpartum remodeling is characterized by decreased hyaluronan (HA) content, increased expression of genes involved in assembly of mature collagen and inflammation. The focus of this study is to advance our understanding of functions HA plays in this dynamic process through characterization of HA size, structure and binding proteins in the mouse cervix. Changes in size and structure of HA before and after birth were observed as well as cell specific expression of HA binding proteins. CD44 expression is localized to the pericellular matrix surrounding the basal epithelia and on immune cells while inter α trypsin inhibitor (IαI) and versican are localized to the stromal matrix. Colocalization of HA and IαI is most pronounced after birth. Upregulation of the versican degrading protease, ADAMTS1 occurs in the cervix prior to birth. These studies suggest that HA has multiple, cell specific functions in the cervix that may include modulation of tissue structure and integrity, epithelial cell migration and differentiation, and inflammatory responses.

Keywords: Parturition; Cervical ripening; Hyaluronan; CD44; Versican

1. Introduction

The molecular mechanisms controlling cervical remodeling during pregnancy and parturition remain to be elucidated and are critical towards the development of therapies to reduce the incidence of preterm birth. The cervix is a collagen-rich structure that must remain tightly closed during pregnancy to keep the fetus in the uterus. In preparation for birth, the cervix undergoes a progressive remodeling throughout pregnancy. The earliest phase of remodeling, termed softening, is characterized by an increase in collagen solubility and tissue distensibilty as compared to nonpregnant cervix (Read et al., 2007). As softening progresses and overlaps with the later phase of cervical ripening, there is increased cell proliferation, tissue hydration and a progressive increase in tissue distensibility without any further increase in collagen solubility (Mahendroo et al., 1999; Read et al., 2007). Cervical ripening and dilation of the cervix upon initiation of uterine contractions is characterized by extensive changes in the structure of the extracellular matrix. Changes in collagen fibril structure brought about in part by changes in glycosaminoglycan and proteoglycan composition result in a loss of tensile strength of the cervical matrix and the ability of the cervix to dilate upon generation of forceful coordinated uterine contractions (Leppert, 1995).

Abbreviations: ITI, inter α trypsin inhibitor protein family; IαI, inter α trypsin inhibitor; PαI, pre-α-trypsin inhibitor; ADAMTS1, a disintegrin and metalloprotease with thrombospondin-like repeats-1; HA, hyaluronan; HC, heavy chain; HAPLN, link protein family; TSG6, TNF stimulated gene 6; RTPCR, real time polymerase chain reaction.

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* Corresponding author. Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-9032, United States. Tel.: +1 214 648 3091; fax: +1 214 648 9242.
E-mail address: mala.mahendroo@utsouthwestern.edu (M. Mahendroo).

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One major change in numerous species during this period is the large increase in synthesis of the glycosaminoglycan, hyaluronan (HA) (Anderson et al., 1991; Downing and Sherwood, 1986; El Maradny et al., 1997; Rajabi et al., 1992). Increased HA synthesis in human and mouse cervix at term results from increased transcription of one of three HA biosynthetic enzymes, hyaluronan synthase 2 (HAS2) (Straach et al., 2005). HAS2 is expressed in the cervical epithelia while the majority of HA is located in the matrix surrounding the cervical stroma.

Hyaluronan (HA) is a polymer made up of repeating disaccharides of D-glucuronic acid and β-1, 3-N-acetylglucosamine-β1,4. HA exhibits unusual physiochemical properties because of its random coil structure, its large size, and its capacity to interact with water. Thus HA forms solutions with high viscosity and elasticity that provide both space-filling, lubricating functions, serves as a substrate for assembly of proteoglycans, promotes cell movement, regulates cell function and development and is involved in tumor progression, inflammation and wound healing (Almond, 2007; Lee and Spicer, 2000; Tammi et al., 2002).

HA functions are determined in part by the size of HA, structure of HA and HA's interaction with HA binding proteins termed hyaladherins. High molecular weight HA plays a structural role and promotes tissue integrity while low molecular HA may be a signal of tissue injury or wound healing (Almond, 2007). The association of HA with specific hyaladherins can modulate the physical structure and properties of HA within the ECM. For example, viral infection of colon smooth muscle cells or inflammation of the colon result in formation of HA cables structures which are highly adhesive for mononuclear leukocytes (de la Motte et al., 2003). A similar observation has been made in renal proximal tubular epithelial cells (Selbi et al., 2006). In contrast, HA structures described as aggregates or coats are not adhesive for tissue monocytes. The formation of cable-like filamentous structures requires the binding of HA to the heavy chain (HC) subunit of the inter α trypsin inhibitor protein family (ITI). Molecules of the ITI family are composed of a common proteoglycan subunit termed, bikunin (40 kDa) and heavy chains (75 kDa) which are encoded by 3 distinct genes (Salier et al., 1996). One family member, pre-α-trypsin inhibitor (PαI) contains one heavy chain while inter-α trypsin inhibitor (IαI) contains two heavy chains. The heavy chain (HC) is also pivotal in formation of a stabilized HA-rich cumulus oocyte complex during ovulation (Mukhopadhyay et al., 2001; Sato et al., 2001; Zhuo et al., 2001). The transfer of the HC to HA is catalyzed by TNF stimulated gene 6 (Tsg6) in the ovary but is not required for transfer of HC to HA in the renal proximal tubular epithelial cells (Fulop et al., 2003; Selbi et al., 2006).

The association of hyaluronan with HA-binding proteoglycans such as versican, aggrecan and brevican is essential to the stabilization of HA structure and assembly of several tissues during development and postnatal life. HA interactions with proteoglycans are further strengthened by associations with members of the link protein family (HAPLN 1–4) (Spicer et al., 2003). For example, HA forms aggregate structures with aggrecan which provide stable load bearing capabilities to cartilage. Other HA binding proteins include the cell surface receptor, CD44, which mediates cell signaling of HA and internalizes HA for breakdown by hyaluronidases. In addition the assembly and retention of a pericellular matrix of many cells involves interactions between CD44 and HA (Day, 1999).

In the current study hyaluronan size, structure and HA binding proteins that may influence HA function were evaluated during mouse pregnancy and parturition in an effort to better understand the biological roles HA may play in cervical remodeling.

2. Results

Mice at late stages of gestation were utilized to evaluate temporal changes in HA molecular weight. While total hyaluronan content is increased in the cervix during cervical ripening, changes in the distribution of hyaluronan based on estimated molecular size is unknown (Straach et al., 2005). Cervical extracts from gestation days 12, 15, 16, 17, 18, 18.75, in labor (IL) and postpartum samples at 2–4 h pp, 10–12 h pp, 24 h pp were run on agarose gels along with high and low HA molecular weight standards. Gel was stained with Stains-All as previously described and HA is stained in blue (Fig. 1) (Lee and Cowman, 1994). Relatively little HA is present until day 18 when there is an increase in high and to a lesser extent low molecular weight HA with greater amounts by late day 18 (18.75), in labor on day 19 (IL) and 2–4 h postpartum. Ten to 12 h after birth while high MW HA is still abundant there is also an increase in medium to low molecular weight HA in the range of 495–110 kDa. By 24 h postpartum the majority of HA is medium to small MW HA. These results suggest that during cervical ripening and dilation the majority of HA is large molecular weight and that the breakdown of HA to smaller

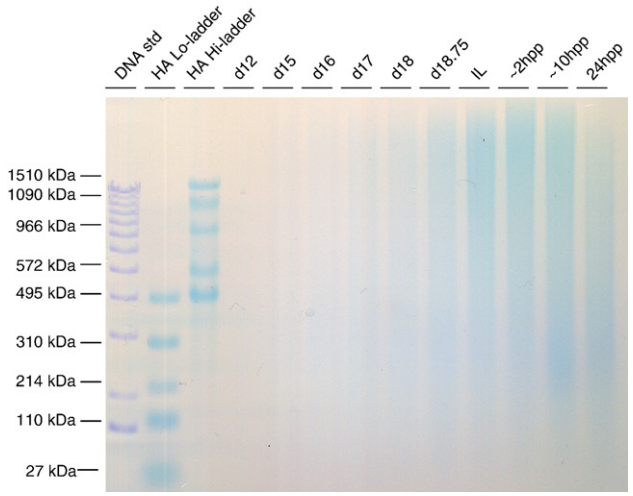

Fig. 1. Assessment of changes in hyaluronan molecular weight during pregnancy, parturition and post partum. Sizes of low and high molecular weight HA standards are indicated to the left of the gel. Equal fractions of cervical extracts from gestation days 12–18.75 (d12–d18.75), in labor (IL), and approximately 2, 10 and 24 h after birth (2 h pp, 10 h pp, 24 h pp) are indicated.