Acta Histochemica xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

MT1-MMP and its potential role in the vertebrate intestinal morphogenesis

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ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form 14 June 2016
Accepted 26 July 2016
Available online xxx

Keywords:
Metalloproteinases
Development
Intestinal mucosa

ABSTRACT

Membrane type 1-matrix metalloproteinase (MT1-MMP) is involved in numerous biological processes, including morphogenesis. However, the role of MT1-MMP in the development of the vertebrate intestine is poorly understood. This study aimed to evaluate the expression of MT1-MMP in the intestine of rats and chickens along the embryonic and postnatal periods using immunohistochemistry. Results revealed a remarkable spatiotemporal correlation between MT1-MMP expression and intestinal villi morphogenesis in both vertebrates. However, the villi morphogenesis process was found to be different in chickens to that of rats. Moreover, extensive MT1-MMP labeling was observed in the entire villus epithelium from birth until the complete maturation of the small intestinal mucosa in both vertebrates. From these results, we suggest that MT1-MMP contributes to intestinal development, particularly to villi morphogenesis, in both vertebrates. However, further studies are necessary to confirm the role of MT1-MMP in this cellular process. In addition, we performed validation of the primary antibody against human MT1-MMP for adult chickens.

1. Introduction

Matrix metalloproteinases (MMPs) comprise a large family of zinc-dependent endopeptidases that are capable of degrading all extracellular matrix (ECM) components. MMPs are involved in pathological processes, such as cancer, vascular diseases, atheroma, arthritis and tissue ulceration (Egeblad and Werb, 2002; Visse and Nagase, 2003; Hu et al., 2007; Zitka et al., 2010), as well as morphogenesis (Alexander et al., 1997; Holmbeck et al., 1999; Kanwar et al., 1999; Legallicier et al., 2001; Longin et al., 2001; Oh et al., 2004; Ayoub et al., 2005; Linask et al., 2005; Kurakata et al., 2008; Yang et al., 2008; Margagliotti et al., 2008; Arnould et al., 2009; Bruni-Cardoso et al., 2010; Aujla and Huntley, 2014; Vos et al., 2014; Kim et al., 2014; Kraljevic et al., 2015; Hasebe et al., 2006). Some studies indicate that MMPs act by remodeling and degrading ECM components to promote proliferation, migration and cell death (Alexander et al., 1997; Ayoub et al., 2005; Linask et al., 2005; Longin et al., 2001; Margagliotti et al., 2008; Bruni-Cardoso et al., 2010; Arnould et al., 2009; Gomes et al., 2010).

Membrane type 1-matrix metalloproteinase (MT1-MMP) is a metalloproteinase, anchored in the cell membrane, which degrades collagen type I, II, III and other ECM components of the basal membrane of epithelial cells. MT1-MMP, complexed with the tissue inhibitor of metalloproteinase 2 (TIMP-2), promotes the activation of the collagenase MMP-2, which then propagates its effects on the ECM, leading to several changes in cellular behavior (Itoh et al., 2001).

Studies of vertebrate morphogenesis have demonstrated that MT1-MMP is important to skeletal formation (Holmbeck et al., 1999; Yang et al., 2008), the vascular system and skeletal muscle maturation (Oh et al., 2004), and organogenesis (Kanwar et al., 1999; Legallicier et al., 2001; Longin et al., 2001; Margagliotti et al., 2008; Janssens et al., 2013; Vos et al., 2014). During rat and chicken intestinal development, numerous changes occur during the morphogenesis of the intestinal epithelium (Dunn, 1967; Mathan et al., 1976; Trier and Moxey, 1979; Madara et al., 1981; Dauça et al., 1990; Vagnerová and Kučera, 2004; Penkova et al., 2010). Molecules studied during intestinal development include bone morphogenic

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http://dx.doi.org/10.1016/j.acthis.2016.07.009
0065-1281/

protein (BMP), Hedgehog, fibroblast growth factor (FGF), Notch, Sox, Wnt and Hox (Barbara et al., 2003; Theodosiou and Tabin, 2003; Mclin et al., 2009; Zorn and Wells, 2009; Pinchuk et al., 2010; Spence et al., 2011; Grosse et al., 2011; Plageman et al., 2011). To the best of our knowledge, few reports have focused on the role of MMPs in the development of the vertebrate intestine. It has been suggested that MMP-2 promotes degradation and remodeling of ECM components during fetal rat colon morphogenesis (Kurakata et al., 2008), while MT1-MMP and gelatinase A (MMP-2) promote degradation or remodeling of ECM associated with the development of adult longitudinal muscle in the intestine during amphibian metamorphosis (Hasebe et al., 2006).

As MT1-MMP is involved in degenerative diseases and the morphogenesis process, we investigated the expression of this protein during the intestinal development of rats and chickens, with the aim of defining its spatiotemporal expression in the intestinal epithelium during the embryonic and postnatal phases. These vertebrates were chosen because they present the same time of embryonic development, 21 days, and similar morphogenetic processes during small intestinal development. We describe herein, for the first time, morphological alterations that occur during chicken intestinal development, as well as the spatiotemporal correlation between MT1-MMP expression and the morphogenesis and maturation of the small intestinal mucosa in both vertebrates.

2. Materials and methods

2.1. Animal samples

2.1.1. Rats

Adult male Wistar rats, from the animal colony of the State University of Ponta Grossa, were mated with adult females in the proportion of 1:3 during the evening period. The vaginal fluid was collected to detect spermatozoids. Pregnant females were separated to collect embryos in the stages of the development described below. All females were maintained under conventional conditions with a 12 h light/dark cycle (lights on at 06:30 h am/lights off at 6:30 h pm) at 25 °C and received food (balanced ration from Nuvital, Brasil) and water *ad libitum*.

2.1.2. Chickens

Fertilized chicken eggs were obtained from Matrizeiro Industrial Idôneo (situated in Carambeí City in the State of Paraná- Brazil). After hygienizing, eggs were maintained in an incubator at constant temperature at 37.8 °C, humidity of 60%, and turned daily. All experiments were approved by the Animal Ethics Committee (CEUA) of the State University of Ponta Grossa.

2.2. Stages of development

The embryonic and postnatal stages of rats and chicken were chosen based on the literature (Dunn, 1967; Dauça et al., 1990; Hiramatsu and Yasugi, 2004; Vagnerová and Kučera, 2004; Penkova et al., 2010). For each evaluation, three animals were used.

2.2.1. Rats

Embryos were collected on the 15th, 17th, 18th and 19th post-fertilization days (named E15, E17, E18 and E19, respectively). Pups were collected at birth and on the 3rd, 10th, 18th and 25th days after birth (named P0, P3, P10, P18 and P25, respectively). Pregnant females and rat pups were briefly anesthetized with halothane (Sigma, USA) and sacrificed by cervical dislocation.

2.2.2. Chickens

Embryos were collected on the 10th, 13th, 16th and 21th days post-fertilization (named E'10, E'13, E'16 and E'21, respectively). The postnatal stages evaluated were at hatching, and on the 3rd, 7th, 10th and 14th days (named P'0, P'3, P'7, P'10 and P'14, respectively). After being briefly anesthetized with halothane (Sigma, USA), chicks were sacrificed by cervical dislocation.

2.3. Histological procedures

The whole embryos of rats or fragments of their small intestine were immersed in 2% paraformaldehyde (SYNTH, USA)/0.1 M phosphate buffer, pH 7.4, for 48 h. The whole embryos of chickens or fragments of their small intestine were submitted to the same procedure. Samples were dehydrated in alcohol and embedded in paraffin to obtain semi-seriated sections of 5 μm. The sections were mounted on slides for immunohistochemistry procedures.

2.4. Primary antibody validation

The primary antibody against human MT1-MMP (Mouse anti-human-MT1-MMP monoclonal antibody, Millipore, USA, cat. No. MAB3317, lot No. LV582533) was validated by Western blot analysis of total protein extracted from adult chicken small intestine (Supplementary material. Fig. S1). Total protein extraction and Western blot were performed as described in Gomes et al. (2010).

2.5. Immunohistochemical procedures

After dewaxing and rehydrating procedures, the sections were quenched three times with 2% hydrogen peroxide for ten minutes each to inhibit endogenous peroxidase activity. Slides were washed in water and 1XPBS, pH 7.4, and they were incubated overnight at 4 °C with mouse anti-human-MT1-MMP monoclonal antibody (purchased from Millipore, USA, cat. No. MAB3317, lot No. LV582533) in 1XPBS, pH 7.4, containing 1% BSA (1:500). Sections were washed in 1XPBS, pH 7.4, and incubated with secondary antibody using Universal LSAB kit (purchased from DAKO, USA, cat. No. K0690, lot No. 10059904) for thirty minutes at 37 °C. After washing in 1XPBS, pH 7.4, sections were incubated with 3% DAB reagent (SIGMA, USA) prepared in 1XPBS, pH 7.4, in the presence of 1.25% hydrogen peroxide and 1.25% DMSO (SIGMA, USA) for 10 min. Negative control staining was prepared by either omitting the primary antiserum or substituting the primary antiserum by non-immune serum.

For analysis of spatial and temporal MT1-MMP immunoreactivity, the slides were photographed by Olympus DP72 microscopy and images of nine sections of each animal at the different developmental stages were evaluated qualitatively (Wise et al., 1992). For each animal, the level of MT1-MMP immunoreactivity, presented in each intestinal phase, was analyzed by two different researchers, using a double blind approach. The comparative analysis among the phases and regions of the small intestine in the morphogenesis process allowed us to determine the level of immunoreactivity as absent (−), weak (+), moderate (++) and strong (+++), at a magnification of 50×.

3. Results

3.1. Spatiotemporal pattern of MT1-MMP in the small intestinal mucosa during rat development

3.1.1. Embryonic development

At E15, MT1-MMP immunoreactivity was weak in the pseudostratified epithelium lining the primary lumen (Fig. 1A). By E17, it was possible to observe epithelial cells (transitional epithelium) in the process of reorganization to form a simple columnar epithelium. Moreover, secondary lumina due to cell adhesion disruption were observed, indicating that the villi morphogenesis had started.

Please cite this article in press as: Camargo, K.C., et al., MT1-MMP and its potential role in the vertebrate intestinal morphogenesis. Acta Histochemica (2016), http://dx.doi.org/10.1016/j.acthis.2016.07.009