Journal of Oral Biosciences 55 (2013) 23–28

Contents lists available at SciVerse ScienceDirect

Journal of Oral Biosciences

journal homepage: www.elsevier.com/locate/job

Review

Role of perlecan, a basement membrane-type heparan sulfate proteoglycan, in enamel organ morphogenesis

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ARTICLE INFO

Article history:
Received 1 November 2012
Received in revised form
28 November 2012
Accepted 5 December 2012
Available online 12 February 2013

Keywords:
Perlecan
Stellate reticulum
Enamel organ
Heparanase
Heparan sulfate

ABSTRACT

Perlecan is a multifunctional heparan sulfate proteoglycan that controls cell-signaling events by interacting with several growth factors, cytokines, and other signaling molecules. Perlecan was thought to be localized only in the basement membrane, but recently, intraepithelial localization of perlecan has been demonstrated in some pathophysiological situations. Therefore, perlecan is expected to modulate epithelial cell behavior. Our recent study demonstrated that perlecan accumulates in the stellate reticulum of the enamel organ of murine molar tooth germs in a stage-specific manner. To understand the function of perlecan in the enamel organ, we generated transgenic (*Tg*) mice that overexpressed perlecan in epithelial cells by using the keratin 5 promoter. Perlecan *Tg* molars had dull-ended crowns and outward-curved tooth roots, and their enamel was poorly crystallized. The constant overexpression of perlecan and the accompanying disorganized distribution of perlecan-related molecules in the enamel organ resulted in irregular tooth morphology. These results indicate that the time schedule of intraepithelial perlecan expression appears to be critically controlled during enamel organ development. In this brief review, we have described the dynamics of perlecan and its receptors and the timing of cleavage of heparan sulfate chains in odontogenesis, focusing on enamel organ development, and discussed the role of perlecan in enamel organ morphogenesis.

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1. Introduction

Tooth development originates with local thickening and invagination of the oral epithelium into the underlying neural crest-derived ectomesenchyme, followed by formation of the enamel organ, dental papilla, and dental follicle. During these processes, extracellular matrix (ECM) molecules play important roles in epithelial–mesenchymal interactions by mediating cellular signal transduction [1–3]. Proteoglycans are one of the primary ECMs and regulate tooth morphogenesis by interacting with various bioactive factors, such as other ECM proteins, growth factors, and adhesion molecules [3–5]. Heparan sulfate proteoglycans (HSPGs)

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http://dx.doi.org/10.1016/j.job.2012.12.004

are composed of a core protein containing one or more linked heparan sulfate (HS) chains and are classified into three groups depending on their localization: (1) syndecans, transmembrane type; (2) glypicans, glycosylphosphatidylinositol (GPI)-anchored type; and (3) perlecan, agrin, and collagen XVIII, extracellular/secreted (basement membrane) type These HSPGs play crucial roles in cell and tissue assembly during organogenesis [6,7]. Especially in odontogenesis, syndecans [8–10] and perlecan [11–13] have been demonstrated to control cell differentiation and morphogenesis during murine tooth development.

Perlecan, a basement membrane-type HSPG, is a macromolecule originally identified in the basement membrane [14]. It exists predominantly in basement membranes and ECMs [15–17], but recently, perlecan has been found to accumulate within epithelial tissues in pathophysiological conditions [18–20]. The *in situ* intraepithelial deposition of perlecan is substantial in tumor cell nests of ameloblastomas [18] and in oral squamous cell carcinomas [19], both of which are histologically characterized by their wide intercellular spaces filled with myxoid materials. Moreover, perlecan-deficient keratinocytes have been shown to form a strikingly thin and poorly organized epidermis and to fail to complete their stratification program [20]. These lines of evidence suggest that perlecan regulates the survival, growth, and differentiation of epithelial cells interacting with perlecan-binding soluble factors within epithelial tissue. Therefore, perlecan is also expected to modulate epithelial cell behavior in odontogenesis.

In this review, we focus on perlecan as a major constituent molecule of the intraepithelial stroma of the enamel organ. We describe the dynamics of perlecan and its receptors, as well as the timing of cleavage of heparan sulfate chains from the perlecan core protein in enamel organ development, and discuss the role of perlecan in enamel organ morphogenesis.

2. Structure and function of perlecan

Perlecan is composed of three major heparan sulfate (HS) side chains and 12 probable N-linked carbohydrates on a large core protein with a molecular weight of approximately 400–500 kDa [14]. This core protein consists of five distinct functional domains that interact with various growth factors, ECM molecules, and signaling molecules, such as fibroblast growth factors (FGFs), bone morphogenetic proteins (BMPs), fibronectin, and sonic hedgehog (Shh) [17,21–23]. Some fragments of the perlecan core protein also show biological activity by themselves. The C-terminal domain V of perlecan, named endorepellin, negatively regulates angiogenesis by disrupting the assembly of actin stress fibers and focal adhesions through α2β1 integrin [24,25]. Domain IV contains a novel peptide sequence, which supports adhesion, spreading, and focal adhesion kinase activation, and has been suggested to act as a "complex cluster of heterotypic interaction sites" that supports ECM assembly [21,26–28]. Therefore, perlecan is thought to play important roles in cellular growth, differentiation, adhesion, and motility in tissue remodeling and homeostasis, regulating not only perlecan-binding bioactive factors but also bioactive fragments of perlecan core protein, which may be modulated by proteolysis [16,17,29].

3. Perlecan receptors

The perlecan core protein binds with α-dystroglycan (α-DG) and integrin β1 in its domain V, and they work as major cell surface perlecan receptors (Fig. 1) [30–32]. Dystroglycan is a heterodimeric integral membrane glycoprotein encoded by a single gene, dystrophin associated glycoprotein (Dag-1) [33]. It

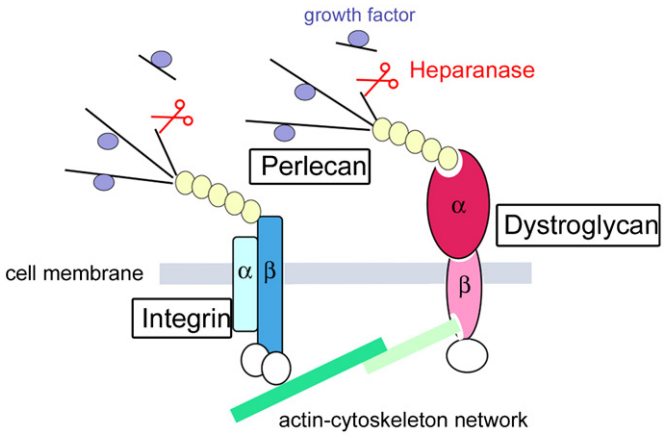

Fig. 1. Schematic representation of perlecan, perlecan receptors, and heparanase. Perlecan core protein binds with α-dystroglycan and integrin β1 on the cell surface. Heparanase cleaves the heparan sulfate (HS) chains from perlecan, and the release of growth factor-bound HS fragments contributes to activating intercellular signaling in surrounding cells.

is composed of α (extracellular) and β (transmembrane) subunits and is bound to major basement membrane components such as perlecan, laminin-1, laminin-2, and agrin [30]. DG is expressed in muscle, neural, and many epithelial cell types [34–36], and it modulates signal transduction pathways and cytoskeleton organization [37,38]. Integrins are the major family of adhesion molecules, and 24 distinct heterodimers, composed of combinations of 18 alpha subunits and 8 beta subunits, are known in mammals [39]. Integrins interact with a wide variety of ECM molecules, and the β1 subunit binds with perlecan, laminin, and type IV collagen [40]. Engagement of integrins activates multiple downstream molecules, which are necessary for cell survival; disengagement of integrin-mediated adhesion to ECM is also required for cellular translocation in tissue morphogenesis and developmental processes [41,42]. Regarding the functional differences between α-DG and integrin β1, Peng et al. have reported that integrins and DG have both been found at the spreading front of lamellipodia on astrocytes *in vitro*; DG contributed to extending cellular processes, while integrins were essential for cellular polarity [32]. With regard to differential distribution of perlecan receptors, we have demonstrated in human ameloblastomas that α-DG is uniformly localized over the stellate reticulum-like cells of ameloblastoma cell nests, whereas integrin β1 is restricted to peripheral cells facing the stromal perlecan at the interface with the basement membrane (Fig. 2). We concluded that integrin β1 in the basal cells of ameloblastoma foci might contribute to cell proliferation, whereas DG in stellate reticulum-like tumor cells might be devoted solely to cellular differentiation by transmitting perlecan signals in a site-specific manner [43].

4. Metabolism of heparan sulfate chains

Heparan sulfate (HS) chains interact with various functional proteins, such as heparin-binding growth factors, cytokines, and ECM molecules, by their negative charges [22,44,45], and thereby function in the control of diverse pathophysiological processes. Another important character of HS chains is that they are capable of retaining water molecules around the straight chains of disaccharide units [46]. Thus, HS chains ensure that a wide variety of bioactive molecules bind to the cell surface and ECM, and thereby function in the control of diverse pathophysiological processes. Heparanase, an endo-β-glucuronidase, cleaves between the uronic acid and glucosamine of HS glycosaminoglycans and