Gene Expression Patterns 13 (2013) 57–65

Contents lists available at SciVerse ScienceDirect

Gene Expression Patterns

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

Expression of multiple delta-protocadherins during feather bud formation

Juntang Lin [a,b,*], Congrui Wang [a,b], Christoph Redies [a]

[a] Institute of Anatomy I, University of Jena School of Medicine, Jena University Hospital, Teichgraben 7, D-07743 Jena, Germany
[b] Xinxiang Medical University, Jinsui Road 601, 453003 Xinxiang, China

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 30 December 2012
Accepted 3 January 2013
Available online 11 January 2013

Keywords:
Delta-protocadherins
Gene expression
Chicken
Feather bud
Pattern formation

ABSTRACT

The expression of the chicken delta-protocadherin (Pcdh) subfamily was investigated in the developing feather buds of the chicken. The expression profiles of the eight investigated *Pcdhs* in the cells and tissues of the feather buds differ from each other. *Pcdh1*, *Pcdh7*, *Pcdh8* and *Pcdh10* are differentially expressed in the epidermis of the feather bud. Expression of *Pcdh1* and *Pcdh10* is restricted to the periderm and *Pcdh17* expression to the epidermis of interbud region. *Pcdh19* is mostly expressed at the anterior side of epidermis as well as in the blood vessels of the feather buds. Furthermore, *Pcdh9* and *Pcdh18* both are regionally expressed in the dermis of the feather bud. These results suggest that Pcdhs may play a variety of roles during avian feather bud formation.

Avian feathers represent an integumental system adapted to a great variety of biological needs (Meyer and Baumgärtner, 1998). The morphogenesis of feather buds is regulated through an interplay of signals that are exchanged between the dermis (mesenchyme) and the epidermis (epithelia); it represents an accessible model to investigate gene functional activity, signal transduction, and the molecular interaction of skin components (Houghton et al., 2003; Jiang et al., 2011). The orientation of the anterior-posterior axis and branches in the feather buds are spatially and temporally regulated by diverse molecular signals (Atit et al., 2003; Chang et al., 2004; Kim et al., 2005; Lin et al., 2006a,b; Drew et al., 2007; Baker et al., 2009; Lin et al., 2011).

During feather bud formation, cell density in the dermis increases initially at a fixed position within the presumptive feather tract; this process then spreads through it (Houghton et al., 2003). Bud and interbud regions form in response to inductive signals from the dermis. Dermal cells are then induced to migrate and form the dermal condensation, and interactions between the epidermis and the dermis determine further bud development (Houghton et al., 2003; Jiang et al., 2011). The molecules that are involved in feather bud formation, either through a positive or a negative regulatory mechanism, include growth factors, homeobox genes, cell adhesion molecules, extracellular matrix molecules like matrix metalloprotease, and transcriptional factors (Chuong, 1993; Widelitz et al., 1999; Dohrmann et al., 2002; Houghton et al., 2003; Rouzankina et al., 2004; Obinata and Akimoto, 2005; Drew et al., 2007; Michon et al., 2008; Baker et al., 2009; Jiang et al., 2011; Lin et al., 2011). Interestingly, bone morphogenetic protein (BMP) family members inhibit embryonic feather formation, and increased expression levels of BMP12 coincide with a naked neck in chicken (Mou et al., 2011). Moreover, a recent study revealed that a nonsense mutation in fibroblast growth factor 20 is associated with the scaleless line of featherless chicken (Wells et al., 2012). To understand the process of bud formation better, we identify members of the delta-protocadherins (Pcdhs) subfamily of cadherins as additional molecular players that are expressed during feather bud formation.

Cadherins are calcium-dependent transmembrane proteins, which form a large family of cell–cell adhesion molecules. Cadherins have been classified into several subfamilies according to sequence similarities in their extracellular as well as cytoplasmic domains. These subfamilies include the classic cadherins, desmosomal cadherins, Flamingo cadherins, fat-like cadherins and protocadherins (Hirano et al., 2003; Redies et al., 2005; Hirano and Takeichi, 2012).

The expression patterns and functions of cadherins have been well investigated in the nervous system (Redies, 2000; Takeichi, 2007; Hirano and Takeichi, 2012; Lin et al., 2012). Classic cadherins were also reported to play various roles during the formation of the feather bud and/or hair follicle (Peifer, 1998; Kaidoh and Inoué, 2008). So far, only two delta-protocadherins (delta-Pcdhs), Pcdh1

Abbreviations: ADAM, a disintegrin and a metalloprotease; BMP, bone morphogenetic protein; Cdh, cadherin; ISH, in situ hybridization; Pcdh, delta-protocadherin.

* Corresponding author at: Institute of Anatomy I, University of Jena School of Medicine, Jena University Hospital, Teichgraben 7, D-07743 Jena, Germany. Tel.: +49 3641 938511; fax: +49 3641 938512.
E-mail address: jlin@mti.uni-jena.de (J. Lin).

1567-133X/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gep.2013.01.001

and Pcdh19 have been reported to be involved in mouse hair follicle formation (Gaitan and Bouchard, 2006; Redies et al., 2008). However, little is known about the expression of the various Pcdhs during feather bud formation. When we investigated the expression of Pcdhs in the developing chicken spinal cord (Lin et al., 2012), we noted that 8 delta-Pcdhs (*Pcdh1*, *Pcdh7*, *Pcdh8*, *Pcdh9*, *Pcdh10*, *Pcdh17*, *Pcdh18* and *Pcdh19*) are also expressed in the cells and tissues of the feather buds and display distinct spatiotemporal expression patterns that suggest a variety of roles for Pcdhs during feather bud formation. These novel findings are the subject of the present study.

1. Results and discussion

The development of the feathers is an example of epithelial-mesenchymal interaction. During feather bud formation, the first visible sign of an individual feather rudiment is the epidermal placode, a localized thickening in the epidermis. Beneath this structure, cells in the dermis condensate to form the dermal papilla (Bellairs and Osmond, 2005). Gene interactions between the epidermis and underlying dermis play an important role in the initial development of feather buds. Signals from the epidermis result in cell aggregation in the dermis, which in turn, provide signals back to the epidermis (Chuong et al., 1996; Bellairs and Osmond, 2005; Jiang et al., 2011). As cell adhesion molecules, cadherins and their ligands, catenins, have been confirmed to be involved in the feather bud formation (Chuong et al., 1991; Noramly et al., 1999; Houghton et al., 2007). In this study, we demonstrate for the first time that eight members of the delta-protocadherin family are expressed under tight spatial control in developing feather buds of chicken embryos. To date, a chicken ortholog gene of the remaining mammalian delta-protocadherin (Pcdh11) has not been identified.

The expression patterns of the eight *Pcdhs* are displayed in Figs. 1–3. In the figure panels, results for each *Pcdh* are arranged according to developmental stages, from early feather buds to late feather buds. Schematic diagrams of the expression pattern of each *Pcdh* are shown in Fig. 4, respectively. Our results revealed that each of the eight *Pcdhs* displays a characteristic spatiotemporal expression profile that differs from that of the other *Pcdhs*. In this study, hematoxylin and eosin staining was used to visualize the feather bud morphology (HE in Fig. 1A, B), and Hoechst 33258 nu-

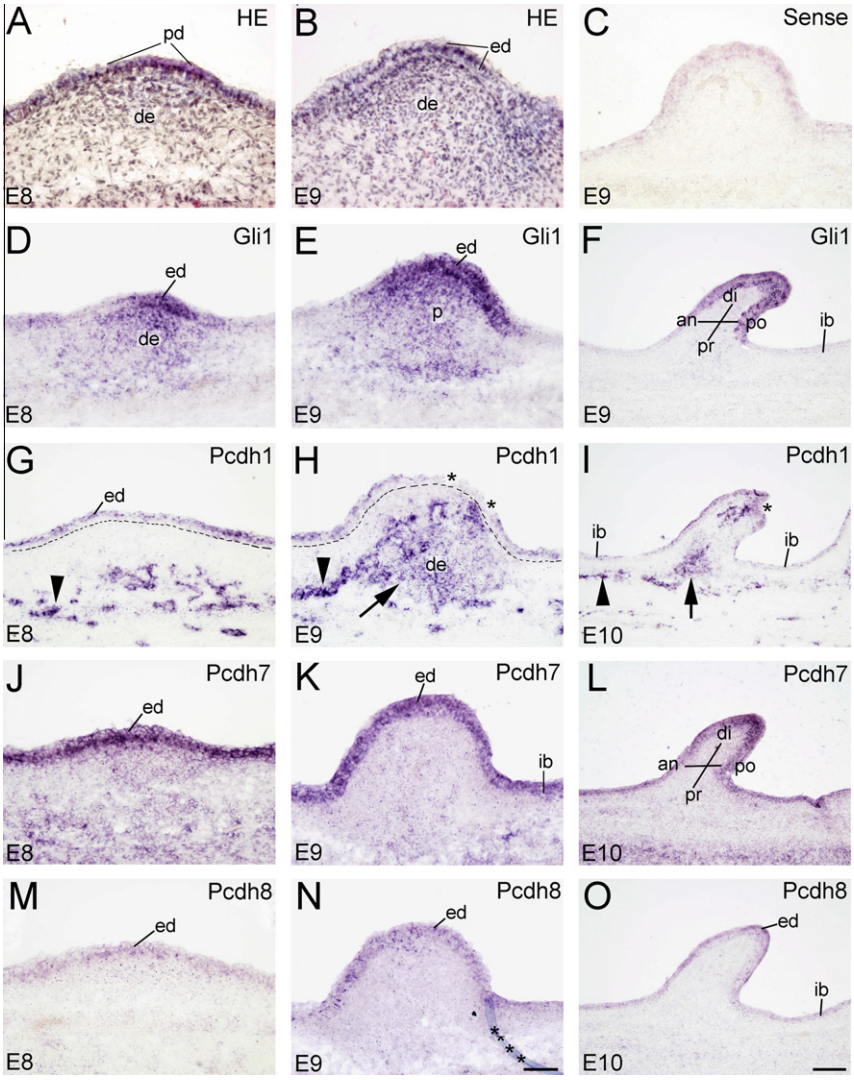

Fig. 1. Expression of *Pcdh1* (G–I), *Pcdh7* (J–L), *Pcdh8* (M–O) during feather bud formation at different days of incubation (E; labeled in each panel), investigated with in situ hybridization. Hematoxylin and eosin staining (HE) shows the morphological structure of the feather bud (A, B). Sense probe for *Pcdh1* served as a negative control (C). Expression of *Gli1* served as a marker for epidermis and the superficial dermis layer in the developing feather buds (D–F). The dotted lines in G and H indicate the boundary between the epidermis and the dermis. Arrowheads in G–I indicate blood vessels. Arrows in H and I point to *Pcdh1*-positive cells in the dermis of the elongated (late) feather buds. Asterisks in H, I and N indicate artifacts. Abbreviations: an, anterior; de, dermis; di, distal; ed, epidermis; ib, interbud region; p, papilla; po, posterior; pr, proximal. Scale bars: 50 μm in N (for A–E, G, H, J, K, M and N); 100 μm in O (for F, I, L and O).