Periostin is required for matricellular localization of CCN3 in periodontal ligament of mice

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Abstract CCN3 is a matricellular protein that belongs to the CCN family. CCN3 consists of 4 domains: insulin-like growth factor-binding protein-like domain (IGFBP), von Willebrand type C-like domain (VWC), thrombospondin type 1-like domain (TSP1), and the C-terminal domain (CT) having a cysteine knot motif. Periostin is a secretory protein that binds to extracellular matrix proteins such as fibronectin and collagen. In this study, we found that CCN3 interacted with periostin. Immunoprecipitation analysis revealed that the TSP1-CT interacted with the 4 repeats of the Fas 1 domain of periostin. Immunofluorescence analysis showed co-localization of CCN3 and periostin in the periodontal ligament of mice. In addition, targeted disruption of the *periostin* gene in mice decreased the matricellular localization of CCN3 in the periodontal ligament. Thus, these results indicate that periostin was required for the matricellular localization

of CCN3 in the periodontal ligament, suggesting that periostin mediated an interaction between CCN3 and the extracellular matrix.

Keywords Periostin · CCN3 · Periodontal ligament

Abbreviations

BMP	Bone morphogenetic protein
CT	Carboxyl-terminal (C-terminal) domain
EMI	EMILIN
Fas 1	Fasciclin I
IGFBP	Insulin-like growth factor-binding protein
TSP1	Thrombospondin type 1
VWC	von Willebrand factor type C

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Introduction

Matricellular proteins that are secreted into the extracellular milieu are localized on the surface of cells and/or extracellular matrix to regulate cell states by interacting with cell-surface receptors, cytokines, and hormones (Bornstein 2009). Matricellular proteins are ubiquitously expressed during embryogenesis, and are also expressed in injured tissues in adulthood (Bornstein 2009; Chatterjee et al. 2014; Krupska et al. 2015). These proteins include SPARC, BM-40, myocilin, thrombospondin-1 (TSP-1), TSP-2, osteopontin, tenascins, β ig-h3 (TGFB1), CCN family, and periostin (Norris et al. 2009; Kudo 2011; Conway et al. 2014; Chatterjee et al. 2014; Krupska et al. 2015).

The CCN family is composed of 6 proteins, CCN1 to 6 (Perbal 2013; Krupska et al. 2015), each of which is expressed in different tissues (Li et al. 2015). CCN3, also known as NOV (Nephroblastoma Overexpressed), is expressed in osteoblasts, smooth muscle cells, nerve cells, and tumor cells (Su et al. 2001; Ouellet and Siegel 2012; Krupska et al. 2015; Li et al. 2015). CCN3 is N-glycosylated and secreted into the extracellular milieu as a dimer, becoming localized on the cell surface and extracellular matrix (Joliot et al. 1992; Chevalier et al. 1998; Kyurkchiev et al. 2004; Perbal 2006, 2013). CCN3 interacts with extracellular matrix proteins such as Fibulin-1C (Perbal et al. 1999) and cell-surface heparan sulfate proteoglycans (Ouellet and Siegel 2012).

The pathophysiological roles of CCN3 have been demonstrated in *Ccn3*^{-/-} mice. *Ccn3*^{-/-} mice exhibit a mild skeletal phenotype (Canalis et al. 2010) and a hyper-proliferative response to vascular injury (Leask 2010; Shimoyama et al. 2010). In the murine model of abdominal aortic aneurysm, *Ccn3*^{-/-} mice exhibit severe phenotypes characterized by elastin fragmentation, vessel dilation, vascular inflammation, dissection, heightened ROS generation, and smooth muscle cell loss, suggesting that CCN3 is a regulator of abdominal aortic aneurysm (Zhang et al. 2016). *Ccn3*^{-/-} mice also show metabolic phenotypes such as improved glucose tolerance and insulin sensitivity on a high-fat diet, suggesting that CCN3 can act as an adipocytokine (Martinerie et al. 2016). Thus, CCN3 is an attractive target for therapies of several diseases (Boehm 1989; McCallum et al. 2012; Van Roeyen et al. 2012; Riser et al. 2014, 2015; Li et al. 2015). CCN3 also acts as an anti-fibrotic factor (Abd El Kader et al. 2013; Riser et al. 2014; Leask 2015; Riser et al. 2015). On the other hand, in *Ccn3*^{-/-} mice, interstitial renal fibrosis is reduced in a chronic kidney disease model, suggesting CCN3 as a fibrotic mediator (Marchal et al. 2015). Therefore, the molecular function of CCN3 regarding the progression of fibrosis is controversial (Abd El Kader et al. 2013).

CCN3 has been demonstrated to activate the Notch signaling pathway. CCN3 regulates osteoblastic differentiation via

Notch signal activation (Ouellet and Siegel 2012). In osteosarcoma, CCN3 is highly expressed and correlated with metastasis and poor prognosis (Manara et al. 2002; Perbal et al. 2008). In addition, Notch is linked to metastasis and a worse prognosis in osteosarcoma patients (Hughes 2009). In Notch signaling, extracellular CCN3 is a key regulator: CCN3 interacts with the extracellular domain of Notch 1 and induces its cleavage (Sakamoto et al. 2002; Minamizato et al. 2007). The released Notch intracellular domain localizes into nuclei and acts there as a transcription factor (Schroeter et al. 1998). Thus, the matricellular localization of CCN3 is important for cellular regulation via Notch signaling.

Periostin is expressed in adult tissues such as periodontal ligament, periosteum, and cardiac valves (Kudo 2011). Targeted disruption of the *periostin* gene in mice leads to non-lethal phenotypes such as eruption disturbance of their incisors and disrupted collagen fibrillogenesis (Kii et al. 2006, 2010). Nevertheless, expression of periostin is markedly induced during wound healing, myocardial infarction, and also in cancer stroma (Kikuchi et al. 2008; Shimazaki et al. 2008; Nishiyama et al. 2011; Kikuchi et al. 2014). *Periostin*^{-/-} mice exhibit severe phenotypes in these situations (Shimazaki et al. 2008; Nishiyama et al. 2011; Kikuchi et al. 2014). Periostin binds to the cell surface (Horiuchi et al. 1999) and also to components of the extracellular matrix, such as fibronectin, collagen, and tenascin-C (Takayama et al. 2006; Kii et al. 2010). Immuno-electron microscopic analysis also showed that periostin is localized on the collagen fibrils in the periodontal ligament and heart valve, as well as on the cell surface of fibroblasts in the colon (Kii et al. 2006; Norris et al. 2007; Kikuchi et al. 2008).

Earlier, we revealed the functions of periostin in the promotion of localization of extracellular matrix proteins. Periostin interacts with type I collagen, fibronectin, tenascin-C, and laminin γ 2 chain (Kii et al. 2010; Nishiyama et al. 2011; Kii et al. 2016). These interactions allow the extracellular matrix proteins to be located in the appropriate locus (Kii et al. 2010; Nishiyama et al. 2011; Kii et al. 2016). This appropriate localization of fibronectin, tenascin-C and laminin γ 2 chain proteins plays important roles in mechanical adaption and cell proliferation (Kii et al. 2010; Nishiyama et al. 2011).

Previously, in the purification of periostin from the culture supernatant of MC3T3-E1 cells, we had found that CCN3 co-purified with periostin (unpublished results). In this present study, we revealed the interaction between CCN3 and periostin, and identified the binding domains of these proteins. Periostin deficiency in mice resulted in decreased extracellular localization of CCN3 in the periodontal ligament. Thus, this study suggests that periostin was required for the matricellular localization of CCN3.

Materials and methods

Mice

Care and experiments with animals were in accordance with the guidelines of the Animal Care and Use Committee at Tokyo Institute of Technology. The wild-type C57BL6 mice were purchased from CLEA Japan, Inc. (Tokyo, Japan). Generation of C57BL6 background *periostin*^{-/-} mice was described previously (Kii et al. 2006; Shimazaki et al. 2008).

Plasmid

The HA tag in the pCAGIPuro-periostin-HA (Kii et al. 2010) was replaced with the FLAG tag by the standard PCR method. The ΔEMIΔCT-FLAG, CCN3-FLAG, and CCN3-d5-HA expression vectors were newly constructed with the following primers: for ΔEMIΔCT-FLAG, 5'-ACCGAATTCAAGCTGCAGATTTGAAAGATCTCC-3' and 5'-ACCGATCCGCGGCCGCTTACTACTTGTCATCGTCGTCCTTGTAAGTCTGCTGGATAGAGGAGTTTGTCC-3'; for CCN3-FLAG, 5'-ACCGGATCCCTCGAGGCCACCATGAGCCTCTTCCTGCGAAAGC-3' and 5'-ACCGATCCGAATTCGCGGCCGCTTACTACTTGTCATCGTCGTCCTTGTAAGTCAATTTCTCCTCTGCTTGCTTCAGC-3'; for CCN3-d5-HA, 5'-ACCGGATCCCTCGAGGCCACCATGAGCCTCTTCCTGCGAAAGC-3' and 5'-ACCGGATCCGAATTCGCGGCCGCTTACTAAGCGTAGTCTGGGACGTCGTATGGGTAAGAGACTTCAACTCCTACGG-3'. The other constructs used were described previously (Sakamoto et al. 2002; Tanabe et al. 2010; Kii et al. 2010). The protein tags, FLAG, HA, and human Fc (hFc), were placed in-frame at the C-terminus. All expression vectors were based on the pCXN2, pCAGIPuro, which was provided by Dr. Niwa (Niwa et al. 1998) or on the pCAGIHygro backbone, in which the puromycin resistance cassette of pCAGIPuro was replaced with the hygromycin resistance one. Full details will be provided upon request.

Cell culture and transfection

HEK293T cells were obtained from the Cell Bank of RIKEN BioResource Center and cultured in low-glucose Dulbecco's modified Eagle's medium (Nacalai Tesque, Inc., Kyoto, Japan) supplemented with 10% FBS (MultiSer™; Thermo Fisher Scientific Inc., Beverly, MA or JRH Biosciences, Inc., Lenexa, KS), 100 U/ml penicillin G, and 100 μg/ml streptomycin. One day before transfection, 2.4×10^5 cells or 1.0×10^6 cells were plated in 12-well plates or 3.5 cm dish, respectively. After having been grown to 90% confluence, the cells were transfected with expression vectors by using polyethylenimine (Sigma-Aldrich, St. Louis, MO), as previously described (Boussif et al. 1995) or with Lipofectamine

2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions.

Immunoprecipitation

Cells in 12-well plates or 3.5-cm dishes were scraped on ice into PBS and lysed in 200 μL or 600 μL of lysis buffer (100 mM Tris, pH 7.4, containing 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% TritonX-100, 0.5% NP-40, 1 μg/mL leupeptin and 0.5 mM PMSF), respectively. For immunoprecipitation of HA- or FLAG-tagged protein, the clarified extracts were incubated at 4 °C for 30 min with anti-HA antibody-conjugated agarose (Sigma-Aldrich) or anti-FLAG antibody-conjugated one (Sigma-Aldrich). For immunoprecipitation of hFc-tagged protein, the extracts were incubated with Protein A Sepharose (GE Healthcare Life Sciences, Piscataway, NJ) at 4 °C for 1 h. The bound proteins were eluted in SDS sample buffer (50 mM Tris (pH 6.8), 2% SDS, 0.1% Bromophenol Blue, and 10% glycerol with 50 or 100 mM DTT) with boiling for 5 min.

Western blotting

Samples were separated by SDS-PAGE and blotted onto nitrocellulose membranes. Non-specific binding was blocked by immersion of the membranes for 30 min in 5% skim milk in Tris-buffered saline (TBS) containing 0.1% Tween (TBS-T) at room temperature. The membranes were washed with TBS-T and then incubated at 4 °C for overnight with primary antibodies (Table 1). Signals were detected by using appropriate HRP-conjugated secondary antibodies.

Immunohistochemistry

Wild-type and *periostin*^{-/-} mice were sacrificed under anesthesia, fixed with 4% paraformaldehyde (PFA) in 0.1 M cacodylate buffer (pH 7.4) by perfusion through the cardiac left ventricle. The mandibles from the mice were post-fixed in 4% PFA in PBS at 4 °C for overnight, and then decalcified in 10% EDTA in PBS for 1 month. The mandibles were next embedded in paraffin and sectioned into 4-μm-thick sections. After dewaxing, the sections were pretreated with 0.3% hydrogen peroxide for 20 min, and then with 1% bovine serum albumin (BSA; Serological Proteins Inc.) in PBS for 30 min. They were then incubated with primary antibodies (Table 1) in 1% BSA in PBS at room temperature (RT) for 2 h: anti-RD1 (Shimazaki et al. 2008) or anti-K19 M (Chevalier et al. 1998) (for single-immunostaining) or anti-RD1 and goat anti-CCN3 (for double-immunostaining). After having been rinsed with PBS, the sections were incubated with appropriate secondary antibodies at RT for 1 h. They were then washed with PBS and visualized with diaminobenzidine. Counterstaining was done with methyl green (for single-immunostaining) or DAPI (double-immunostaining).

Table 1 Antibodies used in this study

Antibody	Cat no.	Supplier	Host/clonality	Dilution
FLAG	A4596	Sigma-Aldrich	mouse monoclonal	1:2000 (agarose beads, IP)
FLAG	F7425	Sigma-Aldrich	rabbit polyclonal	1:2000 (W)
GFP	598	MBL	rabbit polyclonal	1:3000 (W)
HA	A2095	Sigma-Aldrich	mouse monoclonal	1:2000 (agarose beads, IP)
HA	H6908	Sigma-Aldrich	rabbit polyclonal	1:2000 (W)
mPeriostin (RD1)	N/A	N/A (raised in our laboratory)	rabbit polyclonal	1:100 (IHC)
mPeriostin (CT)	N/A	N/A (raised in our laboratory)	rabbit polyclonal	1:2000 (W)
hCCN3	AF1640	R&D	goat polyclonal	1:200 (IHC)
hCCN3 (K19 M)	N/A	N/A (gift from Dr. B. Perbal)	rabbit polyclonal	1:150 (IHC)

W Western blotting, IHC Immunohistochemistry, IP Immunoprecipitation. N/A indicates not applicable

Results

CCN3 interacted with periostin

To confirm the co-purification of CCN3 and periostin, we performed a co-immunoprecipitation assay. We co-transfected 293 T cells with the vectors for periostin fused with

human Fc (periostin-hFc) and CCN3 tagged with FLAG (CCN3-FLAG). The cells were lysed and pulled-down with protein A Sepharose. CCN3-FLAG was detected in the co-immunoprecipitated fraction from the lysate of the cells co-expressing CCN3-FLAG and periostin-hFc (Fig. 1a). This result indicated the interaction between CCN3 and periostin.

Fig. 1 CCN3 interacts with the 4 repeats of the Fas 1 domain of periostin. **a** Co-immunoprecipitation assay for CCN3-FLAG and periostin-hFc. 293 T cells were co-transfected with the combinations of CCN3-FLAG, periostin-hFc, and their control vectors. The cell lysates were co-immunoprecipitated with protein A Sepharose. Blot, antibody used for Western blot analysis; IP, antibody used for immunoprecipitation; Input, crude transfected 293 T cell lysates. **b** Domain structures of intact and domain-deleted forms of periostin. All the forms were expressed as HA or

FLAG fusion proteins with the tag at their carboxyl-terminal. The numbers denote the positions of the amino acids of the periostin protein. SS, signal sequence; EMI, EMILIN domain; FAS1, Fasciclin I domain; CT, C-terminal region. **(c, d)** Co-immunoprecipitation assays for CCN3-FLAG and HA-tagged intact or deleted forms of periostin, tagged with HA. 293 T cells were co-transfected with CCN3-FLAG and intact or mutant forms of periostin-HA **(c, d)** or EGFP as a control **(d)**. The total cell lysates were co-immunoprecipitated with anti-FLAG **(c)** or anti-HA **(d)** Agarose

Fig. 2 TSP1 domain of CCN3 interacts with periostin. **a** Domain structures of intact and domain deletion forms of CCN3 protein. All the forms were expressed as HA or FLAG tagged proteins with the tag at their C-terminal. The signal sequence from IgG was fused to the N-terminal deletion constructs (CCN3-d2 and CCN3-d4). The numbers denote the positions of the amino acids of the CCN3 protein. SS, signal sequence; IGFBP, insulin-like growth factor binding protein-like domain; VWC, von Willebrand factor type C-like domain; TSP1, thrombospondin type 1-like domain; CT, C-terminal domain. **b** Co-immunoprecipitation assay for ΔEMIΔCT-FLAG and HA-tagged CCN3-d2, CCN3-d4, or CCN3-d5. 293 T cells were co-transfected with the expression vectors. The total cell lysates were co-immunoprecipitated with anti-FLAG Agarose

CCN3 interacted with the 4 repeats of the Fas 1 domain of periostin

Periostin is composed of an N-terminal signal sequence (SS) and 6 domains: the EMI domain, 4 repeats of the Fas 1 domain,

and C-terminal region (CT; Fig. 1b; Kudo 2011). To clarify the domain of periostin that bound to CCN3, first we constructed various periostin deletion mutants tagged with HA (Fig. 1b). Then we performed a co-immunoprecipitation assay using anti-FLAG Agarose and lysates of 293 T cells co-transfected with the expression vectors for CCN3-FLAG and the intact periostin-HA or its deletion mutants. CCN3-FLAG interacted with all of the periostin-HA mutants (Fig. 1c). To confirm these bindings, we next performed co-immunoprecipitation assay using anti-HA Agarose under the same conditions. Just the same as in Fig. 1c, all of the periostin-HA mutants interacted with CCN3-FLAG (Fig. 1d). These data demonstrate that CCN3 interacted with the 4 repeats of the Fas 1 domain of periostin.

TSP1-CT domain of CCN3 interacted with the 4 repeats of the Fas 1 domain of periostin

CCN3 possesses an N-terminal signal sequence (SS), IGFBP, VWC, TSP1, and CT domains (Fig. 2a; Krupska et al. 2015). To investigate the binding domain of CCN3 to periostin, we designed the CCN3 deletion constructs shown in Fig. 2a. We performed co-immunoprecipitation assay using anti-FLAG Agarose and 293 T cells co-transfected with the expression vectors for the FLAG-tagged 4 repeats of Fas 1 domain (ΔEMIΔCT) and HA-tagged CCN3-d2, CCN3-d4, or CCN3-d5. ΔEMIΔCT-FLAG interacted with CCN3-d2-HA and CCN3-d3-HA, but not with CCN3-d5-HA (Fig. 2b). These data indicate that the TSP1-CT domain of CCN3 interacted with the 4 repeats of the Fas 1 domain of periostin (Fig. 3).

CCN3 co-localized with periostin in the periodontal ligament of mice

We next examined whether CCN3 co-localized with periostin. We performed double-immunofluorescence analysis with thin sections of molars of mice. Immunofluorescent signals for CCN3 were detected as a fibrillar structure in the periodontal ligament (Fig. 4a, d). Signals for periostin were also detected as a fibrillar structure, indicating its matricellular localization (Fig. 4b, e). Almost all of the signals for CCN3 merged with those for periostin (Fig. 4c, f), suggesting that CCN3 interacted with periostin in the periodontal ligament.

Fig. 3 Interaction domains between CCN3 and periostin. Summary of the co-immunoprecipitation assay in 293 T cells described in Figs. 1 and 2. The TSP1-CT domain of CCN3 interacts with the

4 repeats of the Fas 1 domain of periostin. The EMI domain of periostin interacts with extracellular matrix proteins, fibronectin, and type I collagen (Collagen I)

Fig. 4 CCN3 co-localizes with periostin in the mouse periodontal ligament. Fluorescence immunohistochemical analysis of periodontal ligaments of the molars from wild-type C57BL/6 mice. Paraffin sections of mandibles from 8-week-old mice were stained with anti-CCN3 antibody (a, d), anti-periostin antibody (b, e), and DAPI (nuclei, blue signal). Merged images are shown in “c” and “f.” Scale, 50 μ m

Decreased matricellular localization of CCN3 in the periodontal ligament of *periostin*^{-/-} mice

The possible co-localization of CCN3 and periostin on the extracellular matrix encouraged us to examine whether CCN3 would become localized on the extracellular matrix in the absence of periostin. We compared the localization of CCN3 in the molar periodontal ligament (dotted-line boxes in Fig. 5a, b) and dental pulp (dashed-line boxes in

Fig. 5a, b) from wild-type and *periostin*^{-/-} mice. Periostin was expressed in the periodontal ligament (Fig. 5c, Kii et al. 2006), but not in the dental pulp (Fig. 5g, Zhou et al. 2015). In the molar periodontal ligament, periostin was detected in the wild-type tissue (Fig. 5c), but not in the *periostin*^{-/-} tissue (Fig. 5d). Like periostin, CCN3 was also detected as a fibrillar structure as in the molar periodontal ligament from the wild-type mice (Fig. 5e). On the other hand, CCN3 was not detected as a fibrillar structure in the *periostin*^{-/-} tissue (Fig. 5f).

Fig. 5 Decreased matricellular localization of CCN3 in the *periostin*^{-/-} periodontal ligament. Hematoxylin and eosin staining (a, b) and immunohistochemical analysis (c–j) of the mandibles of the mice. Paraffin sections of mandibles from 8-week-old wild-type (a, c, e, g, i) and *periostin*^{-/-} (b, d, f, h, j) mice were stained with hematoxylin and eosin (a, b), anti-periostin (c, d, g, h), or anti-CCN3 (e, f, i, j) antibodies.

High magnifications of dotted-line boxes and dashed-line boxes in “a” and “b” indicate the periodontal ligament (c–f) and the dental pulp (g–j), respectively. Typical immunoreactivities in “c”–“f” are represented as high-magnification images in the insets (white-lined boxes). AB, alveolar bone; D, dentin. Scale, 500 μ m (a, b); 50 μ m (c–f); 20 μ m (insets of c–f); 100 μ m (g–j)

The localization of CCN3 seemed to be inside the cells, not on extracellular matrix. In contrast, in the dental pulp, in which periostin was not expressed (Fig. 5g, h), the localization of CCN3 was not different between the wild-type and the *periostin*^{-/-} tissues (Fig. 5i, j), indicating a possibility that another secretory factor compensated the function of periostin in the dental pulp. Thus, these data indicate that periostin deficiency in mice decreased the matricellular localization of CCN3.

Discussion

This study demonstrated the interaction between the TSP1-CT domain of CCN3 and the 4 repeats of the Fas 1 domain of periostin. Periostin directly binds to the extracellular matrix proteins (collagen I and fibronectin) via its EMI domain (Kii et al. 2010; Fig. 3). This EMI domain is a conserved domain that functions as a protein-protein interaction module, and it is frequently found in secretory and extracellular matrix proteins (Doliana et al. 2000; Callebaut et al. 2003). Thus, this study indicated the possible existence of a CCN3-periostin-extracellular matrix complex. CCN3 was detected on the extracellular matrix in the periodontal ligament, which CCN co-localized with periostin (Fig. 4). Deletion of the *periostin* gene in mice reduced the matricellular localization of CCN3 (Fig. 5e, f), indicating that periostin was required for the matricellular localization of CCN3. The interaction between CCN3 and periostin may have facilitated this matricellular localization.

Our previous study also demonstrated that periostin facilitates the incorporation of tenascin-C into the extracellular matrix and that deletion of the *periostin* gene in mice reduces the tenascin-C content in the extracellular matrix and disrupts the extracellular meshwork structure (Kii et al. 2010). As found presently for CCN3, tenascin-C also interacts with the 4 repeats of the Fas 1 domain (Kii et al. 2010). Thus, periostin may promote the appropriate localization of the matricellular proteins by mediating the interaction between these proteins and the extracellular matrix.

Periostin^{-/-} mice exhibit severe eruption disturbance in their incisors (Kii et al. 2006), which is due to defects in remodeling of the extracellular matrix in the periodontal ligament (Lv et al. 2014). Previously we reported that the expression of cathepsin K, matrix metalloproteinase 1 (MMP1), and MMP2 was significantly decreased in the periodontal ligament of *periostin*^{-/-} mice compared with that in their wild-type counterparts (Lv et al. 2014), suggesting that periostin was required for the expression of these enzymes. CCN3 has been demonstrated to play roles in the expression of MMPs (Laurent et al. 2003; Benini et al. 2005; Lin et al. 2005; Fukunaga-Kalabis et al. 2008; Tzeng et al. 2011; Kular et al. 2012). As examples, the interaction between CCN3 and α

integrin promotes MMP13 expression (Tzeng et al. 2011); and CCN3 mediates the expression of MMP3 via the PDGF signaling pathway (Laurent et al. 2003). These results suggest that CCN3 was partly responsible for the eruption disturbance seen in *periostin*^{-/-} mice.

Periostin is occasionally co-expressed with CCN3 in tissues and diseases, such as periodontal ligament (Fig. 4), aorta (Fu et al. 2009; Zhang et al. 2016), abdominal aortic aneurysm (Yamashita et al. 2013; Zhang et al. 2016), wound healing (Lin et al. 2005; Nishiyama et al. 2011), osteosarcoma (Perbal et al. 2008; Hu et al. 2014, 2016), and vascular injury (Lindner et al. 2005). In these situations, CCN3 plays important roles in the mitigation of abdominal aortic aneurysm (Zhang et al. 2016), the angiogenesis in wound healing (Lin et al. 2005), and the cell migration in osteosarcoma (Huang et al. 2011). Our present data indicated that periostin facilitated the matricellular localization of CCN3. Thus, some parts of the pathophysiological functions of periostin could be explained by CCN3 involvement.

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Author's contributions IT, HT, KK, and AK designed the research. IT, HT, HI, NA, and ML performed experiments. IT, HT, TN, and IK analyzed and interpreted the data. TN and IK wrote the manuscript.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interests.

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