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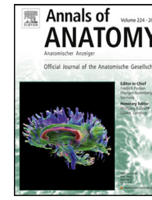

RESEARCH ARTICLE

Class I and Class II small leucine-rich proteoglycans in human cutaneous pacinian corpuscles

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ABSTRACT

Pacinian corpuscles are onion bulb-like multilayered mechanoreceptors that consist of a complicated structure of axon terminals, Schwann related cells (inner core), endoneural related cells (intermediate layer) and perineurial related cells (outer core-capsule). The cells forming those compartments are continuous and share the properties of that covering the nerve fibers. Small leucine-rich proteoglycans are major proteoglycans of the extracellular matrix and regulate collagen fibrillogenesis, cell signalling pathways and extracellular matrix assembly. Here we used immunohistochemistry to investigate the distribution of class I (biglycan, decorin, asporin, ECM2 and ECMX) and class II (fibromodulin, lumican, prolargin, keratocan and osteoadherin) small leucine-rich proteoglycans in human cutaneous Pacinian corpuscles. The distribution of these compounds was: the inner core express decorin, biglycan, lumican, fibromodulin, osteoadherin; the intermediate layer display immunoreactivity for osteoadherin; the outer core biglycan, decorin, lumican, fibromodulin and osteoadherin; and the capsule contains biglycan, decorin, fibromodulin, and lumican. Asporin, prolargin and keratocan were undetectable. These results complement our knowledge about the distribution of small leucine-rich proteoglycans in human Pacinian corpuscles, and help to understand the composition of the extracellular matrix in these sensory formations.

1. Introduction

The Pacini's corpuscles are rapidly-adapting low-threshold mechanoreceptors functionally connected with myelinated Aα or Aβ nerve fibers that detect gross pressure changes and vibrations (Johnson, 2001; Roudaut et al., 2012; Fleming and Luo, 2013; Jones and Smith, 2014). They are widely distributed throughout the body (Bell et al., 1994; Zelená, 1994), and their structure is independent of the anatomical localization (Pawson et al., 2008). Typically they are oval-shaped multilayered capsulated formations that consist of one central axon surrounded by non-neuronal cells arranged in concentric lamellae that form the inner core, intermediate layer, outer core, and capsule (see Bell et al., 1994; Zelená, 1994). The inner core is composed of terminal non-myelinating Schwann-like cells, the intermediate layer is formed by endoneurium-related cells, and the outer core-capsule cells are perineurium-related cells (Munger et al., 1988; Zelená, 1994; Vega et al., 2009; Feito et al., 2016; García-Piqueras et al., 2017). Consistently, the different parts of the Pacinian corpuscles share the immunohistochemical profile of their counterparts in nerves (Vega et al., 1996, 2009). Interposed between the different cell layers forming the Pacinian corpuscles there is an extracellular matrix (ECM) occasionally organized as a basement membrane (BM). Thus, it has been described a BM in relation to the inner core, outer core and capsule of Pacinian corpuscles of different mammalian species (Munger et al., 1988; Bell et al., 1994; Zelená, 1994; Vega et al., 1995; Dubový and Bednárová, 1999). Recently, the presence of chondroitin sulfate proteoglycan chains was found associated to

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Table 1
Primary antibodies used in the study.

Antibody (clone)	Abbreviation	Catalog number – Positive control	Host	Dilution	Source
Primary antibodies to general Pacinian corpuscles markers					
CD34 (QB-END/10)		MAD-001613QD-8	Mouse	Prediluted	A
Neurofilament proteins (RNF402)	NFP	sc-32729	Mouse	1:1000	B
Peripherin		AB1530	Rabbit	1:500	C
PGP9.5		ab10404	Rabbit	1:300	D
S100 protein	S100P	IS504	Rabbit	1:1000	E
S100 protein (4C4.9)	S100P	GTX24066	Mouse	1:2000	F
Vimentin	VIM	sc-7557	Rabbit	1:200	B
Vimentin (V9)	VIM	IS630	Mouse	Prediluted	E
Primary antibodies to SLRPs					
Asporin	ASPN	PA5-13553 – 1	Rabbit	1:200	G
Biglycan	BGN	PA5-13700 – 2	Rabbit	1:500	G
Biglycan (4E1-1G7)	BGN	ab54855 – 3	Mouse	1:100	D
Decorin (9XX)	DCN	sc-73896 – 3	Mouse	1:100	B
Fibromodulin (H-11)	FMOD	sc-166406 – 4	Mouse	1:500	B
Keratocan	KERA	sc-66941 – 5	Rabbit	1:100	B
Lumican	LUM	ab168348 – 4	Rabbit	1:2000	D
Osteoadherin (B-10)	OMD	sc-271102 – 6	Mouse	1:100	B
Prolargin	PRELP	ab103868 – 7	Rabbit	1:100	D
Secondary antibodies					
Anti-rabbit IgG		K4002	Goat	Prediluted	E
Anti-mouse IgG		K4000	Goat	Prediluted	E
Anti-mouse IgG		RE7111	Rabbit	Prediluted	H
Alexa Fluor 488-conjugated anti-rabbit IgG		A11034	Goat	1:1000	I
Cy3-conjugated anti-mouse IgG		715-166-150	Donkey	1:50	J

Source: A: Master Diagnóstica (Granada, Spain); B: Santa Cruz Biotechnology (Santa Cruz, CA, USA); C: Chemicon International (Billerica, MA, USA); D: Abcam (Cambridge, UK); E: Dako (Glostrup, Denmark); F: GeneTex (Irvine, CA, USA); G: Thermo Scientific (Freemont, CA, EEUU); H: Leica-Novocastra Byosistems (Newcastle, UK); I: Invitrogen (Eugene, OR, USA); J: Jackson-ImmunoResearch (Baltimore, MD, USA).
Positive controls: 1: human hepatocarcinoma; 2: human brain; 3: human hyaline cartilage; 4: human kidney; 5: human stomach; 6: human dental pulp; 7: human breast carcinoma. These tissues were obtained from the collection of our laboratory (Registro Nacional de Biobancos, Sección colecciones, Ref. C-0001627, responsible O. G–S).

the intermediate layer in human cutaneous Pacinian corpuscles (García-Piqueras et al., 2018).

ECM is an organized complex network primarily formed of fibrillary proteins, such as collagens and laminins, and proteoglycans (PGs; Iozzo and Schaefer, 2015; Theocharis et al., 2016). Collagens are localized in the interlamellar spaces of Pacinian corpuscles: type II collagen predominates in the outer core and capsule, type V collagen in the intermediate layer, and collagen IV is present in all three extra-neuronal compartments (Vega et al., 1995; Pawson et al., 2000). Conversely, little information is available about the occurrence and distribution of other ECM components, especially PGs, in Pacinian corpuscles.

PGs fall into two main groups: small and large. The group of small PGs (also known as small leucine-rich proteoglycans; SLRPs), contains 18 members that are subclassified into classes I to V. In particular, class I is formed by biglycan (BGN), decorin (DCN), asporin (ASPN), extracellular matrix protein 2 (ECM2) and ECMX, whereas class II is formed by fibromodulin (FMOD), lumican (LUM), prolargin (PRELP), keratocan (KERA) and osteoadherin (OMD) (McEwan et al., 2006; Schaefer and Schaefer, 2010; Chen and Birk, 2013). The members of the small (SLRPs) and large PGs group are in Table 1 of "Supplementary Material". Sames et al. (2001) are the only authors reporting the presence of DCN and BGN in the cat mesentery Pacinian corpuscles.

SLRPs are mainly expressed during neural development but also in adult neural tissues (Le Goff and Bishop, 2007; Dellett et al., 2012). In the peripheral nervous system, including cat Pacinian corpuscles (Sames et al., 2001), some SLRPs have been detected (Hanemann et al., 1993; Wilda et al., 2000). Nevertheless, as far as we know, SLRPs have not been systematically explored in the human Pacinian corpuscles. Thus, the present study was designed to investigate the occurrence and distribution of classes I and II of SLRPs in human digital Pacinian corpuscles, and thus to contribute to the knowledge of ECM composition in sensory corpuscles.

2. Material and methods

Skin samples were obtained from the palmar side of the distal phalanx of amputated fingers (n = 15) collected within 6–8 h after the accident from otherwise healthy subjects (age range 24–42 years). Also, samples of finger tip skin (n = 7; age range 8–56 years) were obtained from necropsies of neurological-disease-free subjects at the Department of Pathology of the Hospital Universitario Central de Asturias (Oviedo, Spain) and Complejo Hospitalario Universitario de Salamanca (Salamanca, Spain). The specimens were fixed in 4% formaldehyde in 0.1 M phosphate buffer saline (pH 7.4) for 24 h, dehydrated, and embedded in paraffin.

These materials were all obtained in compliance with Spanish Law (RD 1301/2006; Ley 14/2007; DR 1716/2011; Orden ECC 1414/2013), were deposited in the collection of our laboratory (Registro Nacional de Biobancos, Sección colecciones, Ref. C-0001627, responsible O. G–S) and the study was approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Cod. CElm, PAst: Proyecto 266/18).

The paraffin-embedded digital skin was cut into 10 μm-thick sections perpendicular to the skin surface and mounted on gelatine-coated microscope slides. The presence of Pacinian corpuscles was ensured staining randomly selected sections with hematoxylin & eosin. Five sections 200 μm apart per skin sample were analyzed, and at least 5 Pacinian corpuscles were evaluated in each specimen.

2.1. Immunohistochemistry

Sections were deparaffinized, rehydrated and rinsed in 0.05 M HCl Tris buffer (pH 7.5) containing 0.1% bovine serum albumin and 0.1% Triton X-100. Thereafter, the endogenous peroxidase activity (3% H_2O_2) and non-specific binding (10% fetal calf serum) were blocked, and the sections were incubated overnight in a humid chamber at 4 °C with the primary antibodies listed in Table 1. The antibodies against protein gene product 9.5 (PGP9.5), neurofilaments proteins (NFP), peripherin, S100 protein, CD34 and vimentin, were used to immunolabel the axon, inner core, intermediate layer and outer core-capsule, respectively, within Pacinian corpuscles (Vega et al., 2009; García-Piqueras et al., 2017). For immunohistochemical detection of BGN the sections were heated in Envision FLEX target retrieval solution of pH6 (Dako) at 65 °C for 20 min and then incubated for another 20 min at room temperature in the same solution, previously to incubation of the primary antibody. After incubation, the sections were rinsed in the same buffer as above and incubated with Dako EnVision System labeled polymer-HR anti-rabbit IgG or anti-mouse IgG (DakoCytomation, Denmark) for 30 min at room temperature. Finally, sections were washed, and the immunoreaction visualized using 3-3′-diaminobenzidine as a chromogen. To ascertain structural details, sections were slightly counterstained with hematoxylin and eosin.

For control purposes, representative sections were processed in the same way as described above using non-immune rabbit or mouse sera instead of the primary antibodies or omitting the primary antibodies in the incubation. Under these conditions, no positive immunostaining was observed (data not shown). Furthermore, positive controls were used to check the specific of the reaction (see Table 1).

2.2. Double immunofluorescence

Based on the results of positivity for SLRPs in simple immunohistochemistry, double immunofluorescence was carried out for DCN or BGN or LUM or FMOD or OMD with axonal markers, S100 protein, vimentin and CD34. Deparaffinized and rehydrated 10 μm thick sections were processed for reduction of non-specific bindings (30 min with a solution of 5% bovine serum albumin in tris-buffer saline –TBS- pH 7,4). Then they were incubated overnight, at 4 °C in a humid chamber with a 1:1 v/v mixture of the selected antibodies. After rinsing with TBS, the sections were incubated for 1 h with Alexa fluor 488-conjugated goat anti-rabbit IgG (Invitrogen, Eugene, OR, USA), diluted 1:1000 in TBS containing 5% mouse serum (Serotec, Oxford, UK), then rinsed again and incubated for another hour with Cy 3-conjugated donkey anti-mouse antibody

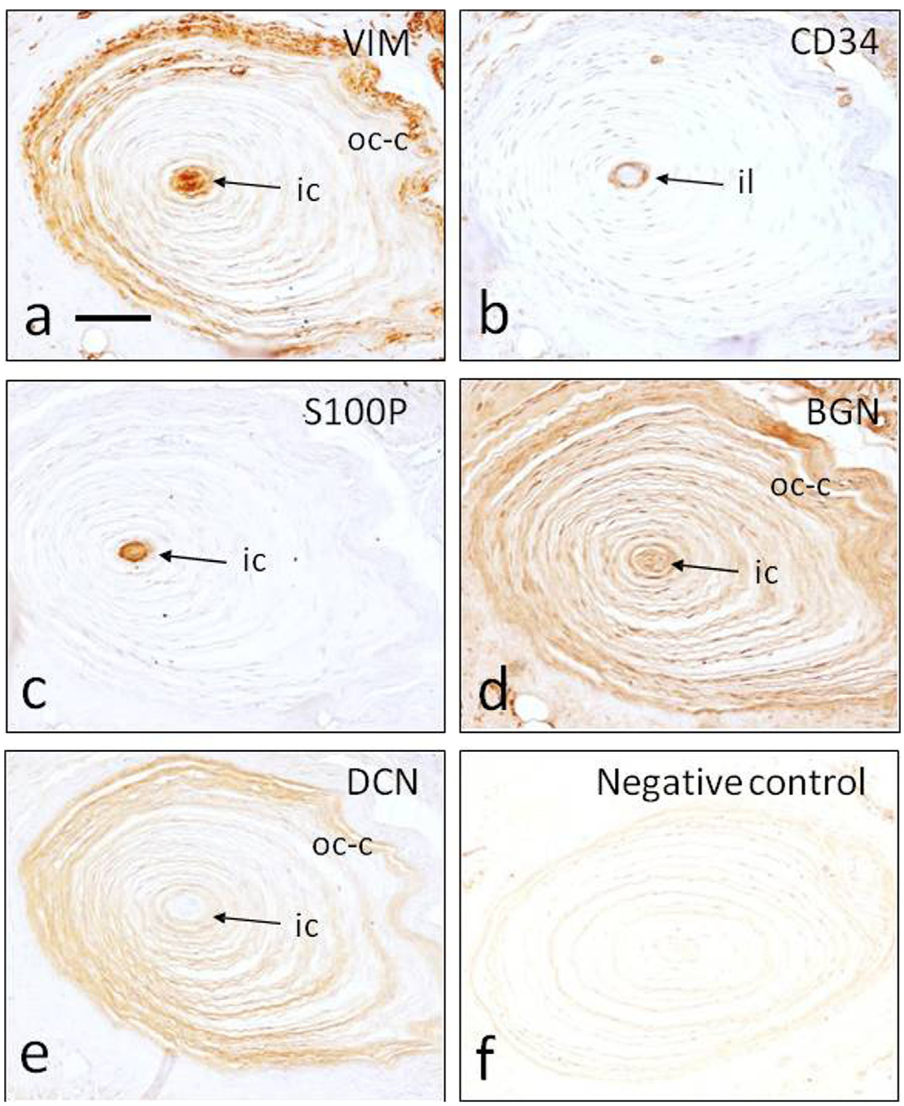

Fig. 1. Serial sections (a–e) of a human digital Pacinian corpuscle showing the immunohistochemical distribution of vimentin (VIM), CD34 and S100 protein (S100 P) used to label the inner core, the intermediate layer, and the capsule (and the inner core), respectively. Biglycan (BGN; d) and DCN (e). Biglycan (BGN) was expressed in all the compartments of Pacinian corpuscles except the axon and the intermediate layer (d). Decorin (DCN) was mainly found in outer-core cells and capsule, in a thin layer between both inner and outer cores and in the S100P-positive inner core (e). Negative control (f). ic: inner core; oc-c: outer core-capsula; il: intermediate layer. Scale bar: 100 μm, a to f. Objective: 20X, a to f.

(Jackson-ImmunoResearch, Baltimore, MD, USA) diluted 1:50 in TBS. Both steps were performed at room temperature in a dark humid chamber. Sections were then washed and mounted with Fluoromount Gold. Finally, to ascertain structural details sections were counterstained with DAPI (10 ng/ml). In the case of double immunodetection of CD34 with DCN, FMOD or OMD, sections were incubated overnight in a humid chamber at 4 °C with primary mouse anti-DCN, anti-FMOD or anti-OMD, followed by incubation with Cy3-conjugated donkey anti-mouse antibody for 90 min. Thereafter samples were incubated overnight with mouse anti-CD34 antibody. After rinsing with TBS, sections were incubated with prediluted rabbit Post-Primary IgG, which recognizes mouse antibodies (Novolink Polymer Detection System®, Leica-Novocastra Biosystems, Newcastle, UK) at room temperature for 90 min. After that, sections were incubated for 90 min at room temperature with Alexa fluor 488-conjugated goat anti-rabbit IgG. Finally, to label nuclei, sections were counterstained with DAPI (10 ng/ml) and mounted with Fluoromount Gold as described above. For control purposes representative sections were processed in the same way as described above using non-immune rabbit or mouse sera instead of the primary antibodies or omitting the primary antibodies in the incubation. Triple staining was detected using a Leica DMR-XA automatic fluorescence microscope coupled with a Leica Confocal Software, version 2.5 (Leica Microsystems, Heidelberg GmbH, Germany) and the images captured were processed using the software Image J version 1.43 g Master Biophotonics Facility, Mac Master University Ontario (www.macbiophotonics.ca).

3. Results

Immunohistochemistry was performed to investigate the occurrence and localization of some members of the class I and class II SLRPs in human digital Pacinian corpuscles. So, we investigated the distribution within them of ASPN (asporin), BGN (biglycan),

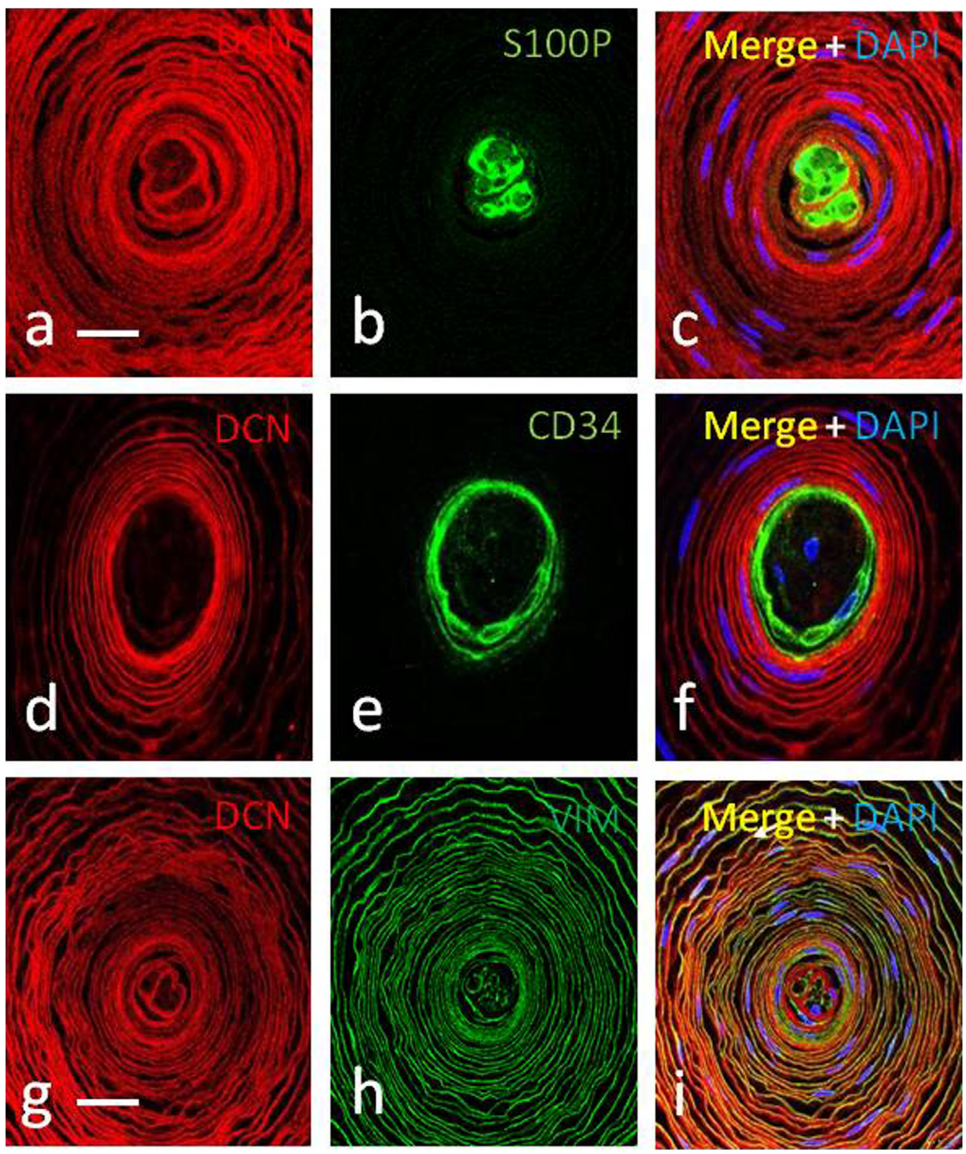

Fig. 2. Double immunofluorescence in Pacinian corpuscles showing the simultaneous localization of decorin (DCN) in red (a, d and g) with S100 protein (S100 P, b), CD34 (e) and vimentin (VIM, h) in green. Although DCN did not colocalize with S100P (a–c), CD34 (d–f) or VIM (g–i), it was found in both inner and outer cores, thus suggesting it is localized in the interlamellar spaces a not in the cytoplasm. In fact, DCN was localized around the inner core cells (a–c), and sometimes the intensity of immunostaining was weak (d). Moreover, a close association between DCN and CD34 or VIM was observed, especially at the periphery of the inner core (d–f) and in the outer core (g–i), respectively. Scale bar: 20 μm, a to f; 40 μm, g to i. Objective: 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm, a to f; 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm, g to i. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

DCN (decorin), FMOD (fibromodulin), OMD (osteoadherin), LUM (lumican), PRELP (prolargin) and KERA (keratocan). As a rule ASPN, PRELP and KERA were undetectable (data not shown) whereas specific immunostaining was detected for the other SLRPs investigated with different patterns of distribution within the corpuscles. We assumed that SLRPs immunoreactivity was always localized around the axon or in the interlamellar spaces, and co-localization with membrane or cytoplasmic proteins is due to image overlapping. On the other hand, slight differences in the intensity of immunostaining were noted among different segments of the corpuscle (preterminal, terminal and ultraterminal) but not with respect to the age of the donors.

3.1. Class I SLRPs

Serial sections of Pacinian corpuscles showed different patterns of distribution for DCN and BGN. In light microscopy DCN and BGN immunoreactivity was observed in all the corpuscular compartments including the intermediate layer, inner and the outer core (Figs. 1a–e). Double immunofluorescence for DCN in combination with specific markers of Pacinian corpuscular constituents (S100 protein, vimentin and CD34) demonstrate that DCN surround the S100 protein positive cells, thus it was localized outside the inner core (Fig. 2a–c). In addition, the relation of DCN immunoreactive structures with the intermediate layer suggests that DCN was also outside this layer (Fig. 2d–f). Therefore, DCN should be expressed in the interlamellar spaces of the outer core and the capsule (Fig. 2g–i).

A strong and specific immunoreactivity for BGN was found in the inner core, outer core and capsule (Fig. 1a–d). However, because of the lack of immunoreactivity observed encircling the inner core, the presence of BGN in the intermediate layer cannot be ensured (Fig. 1d). To ascertain whether BGN is localized in the inner segment of the Pacinian corpuscles simultaneous detection of BGN and S100 protein, or BGN and peripherin, was performed. The results demon-

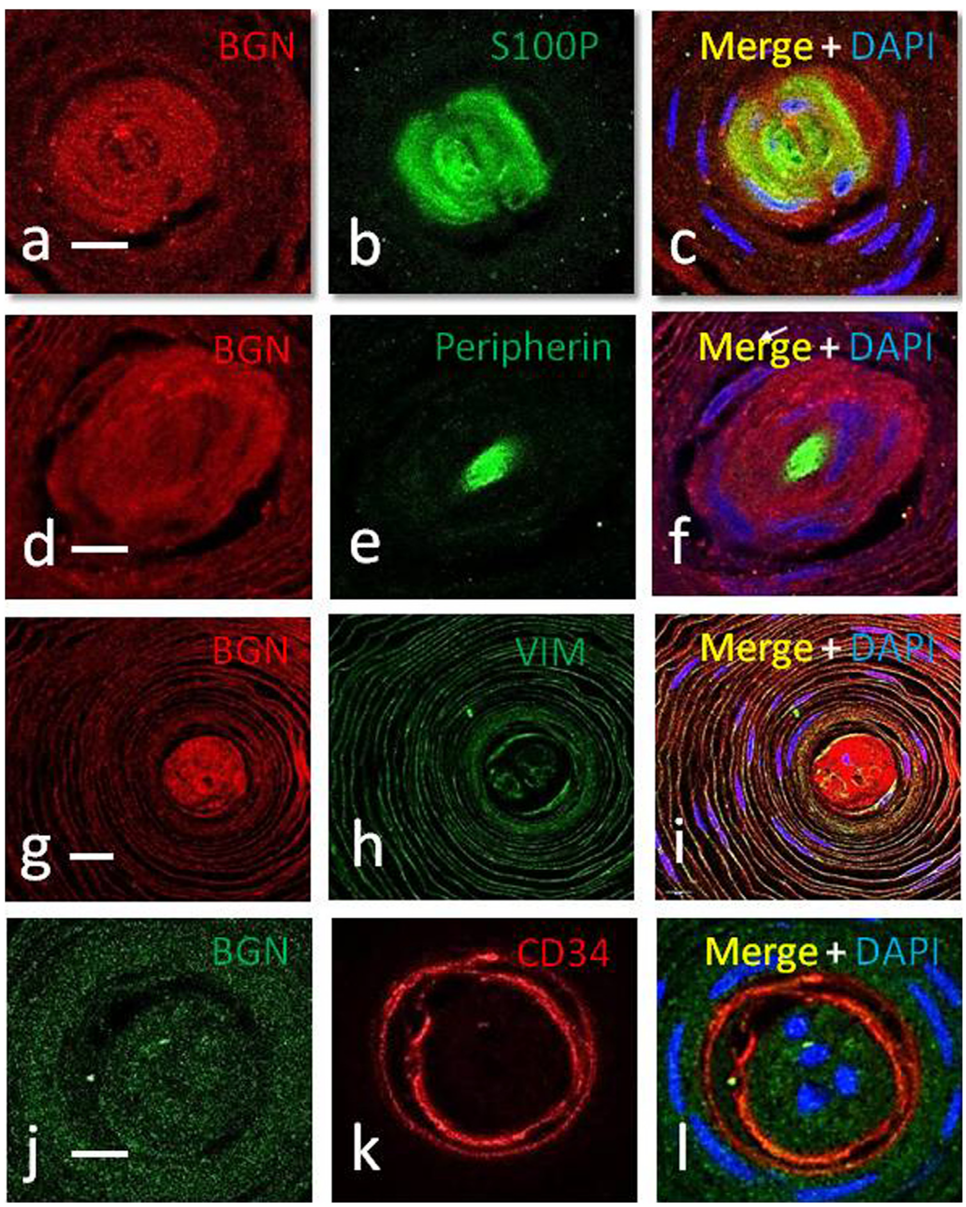

Fig. 3. Double immunofluorescence using for simultaneous localization of biglycan (BGN, a, d, g and j), S100 protein (S100 P, b), peripherin (e), vimentin (VIM, h) or CD34 (k) in Pacinian corpuscles. Expression of BGN was observed in both the inner core (a–c) around the axon (d–f) and the outer core-capsule (g–i). Conversely, BGN was not present in the intermediate layer (j–l). Scale bar: 20 μm, a to c, g to i, j to l; 10 μm, d to f. Objective: 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm, a to f, j to l; 40X, g to i.

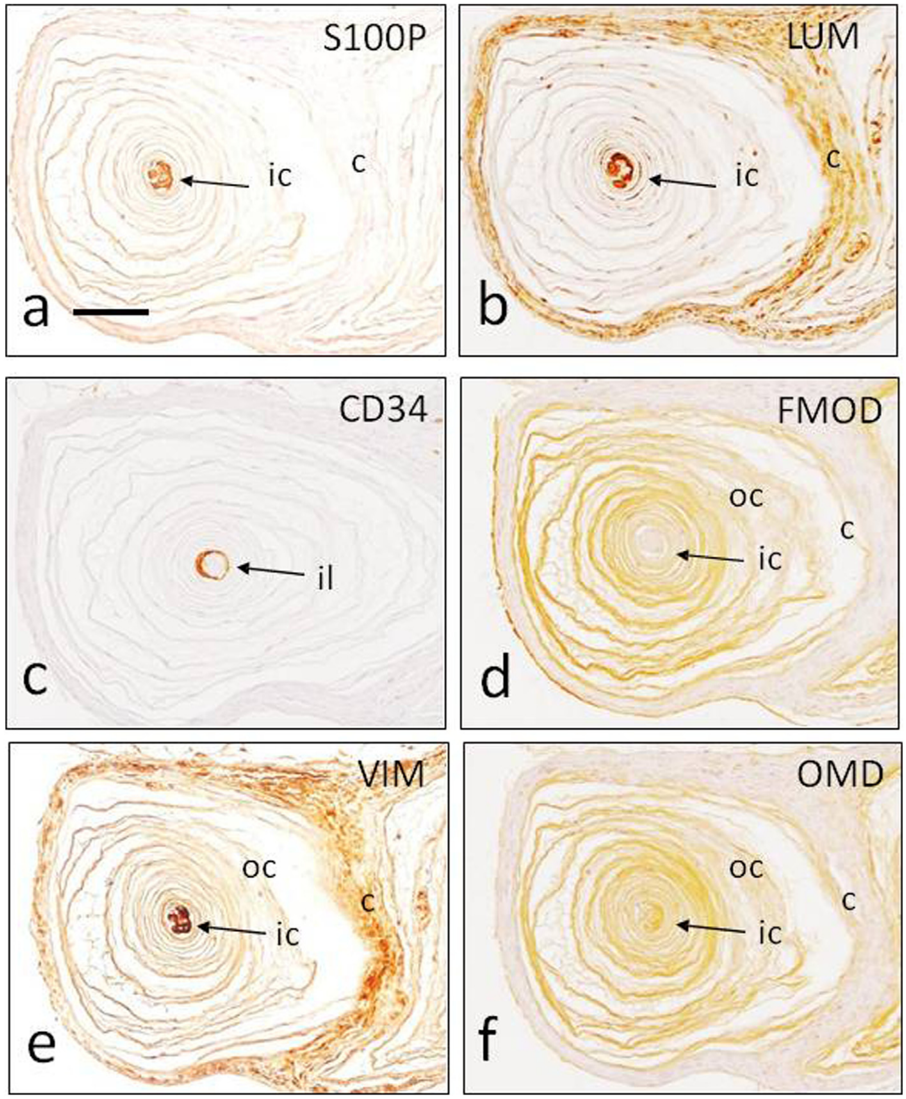

Fig. 4. Immunodetection of S100 protein (S100P, a), lumican (LUM, b), CD34 (c), fibromodulin (FMOD, d), vimentin (VIM, e) and osteoadherin (OMD, f) in consecutive sections of a Pacinian corpuscle using single immunohistochemistry. LUM was found in the inner core, the intermediate layer and the outermost lamellae of the outer core-capsule (b). Both FMOD (d) and OMD (f) were detected in the inner core and outer core, whereas they were segregated in the capsule (OMD) and the intermediate layer (FMOD). ic: inner core; oc: outer core; c: capsule; il: intermediate layer. Scale bar: 100 μm, a to f. Objective: 20X, a to f.

strated that while BGN highly co-localized with S100 protein, thus supporting its presence in the inner core (Figs. 3a–c), it was absent from the central axon (Fig. 3d–f). In addition, co-localization of BGN and vimentin, but not of BGN and CD34, demonstrated that BGN is present in the outer core lamellae and capsule (Fig. 3j-l) but not in the intermediate layer (Fig. 3g-i).

3.2. *Class II SLRPs*

Comparing serial sections processed for detection of S100 P (Fig. 4a), CD34 (Fig. 4c) and vimentin (Fig. 4e) with those used for detection of class II SLRPs, it was apparent that the distribution of LUM immunoreactivity overlaps with the inner core, intermediate layer and the outermost lamellae of the capsule (Fig. 4b); OMD has the same distribution observed with LUM without the capsuleś outermost enhancement (Fig. 4f); FMOD was only detected in the inner core and outer core-capsule (Fig. 4d).

In order to determine the precise localization of these molecules we performed double immunofluorescence with specific antigens of the different corpuscular compartments (Fig. 5). The results of these experiments demonstrated that LUM was absent from the axon (Fig. 5a–c) and the intermediate layer (Fig. 5g–i) while it is localized in the inner core (Fig. 5d–f), and widely in the outer core and capsule (Fig. 5l-k). Regarding FMOD, it was confirmed that FMOD is absent from the axon (Fig. 6akc) and the intermediate layer (Fig. 6g–i). Conversely, a faint but specific reaction was detected in the inner core, although in many cases do not match completely with S100 protein (Fig. 6d–f); the outer core-capsule displayed solid co-localization (Fig. 6j–l). The inner core, intermediate layer and outer core displayed OMD immunoreactivity (Fig. 7). Although in most cases co-localization of OMD and S100P was observed, we have occasionally found that a subpopulation of OMD positive Schwann cells did not express S100 and *vice versa* (Fig. 7c).

Results on the localization of SLRPs in human cutaneous Pacinian corpuscles are summarized in Fig. 8.

4. Discussion

ECM is composed by network of secreted proteins surrounding cells, either as an interstitial matrix or organized into basement

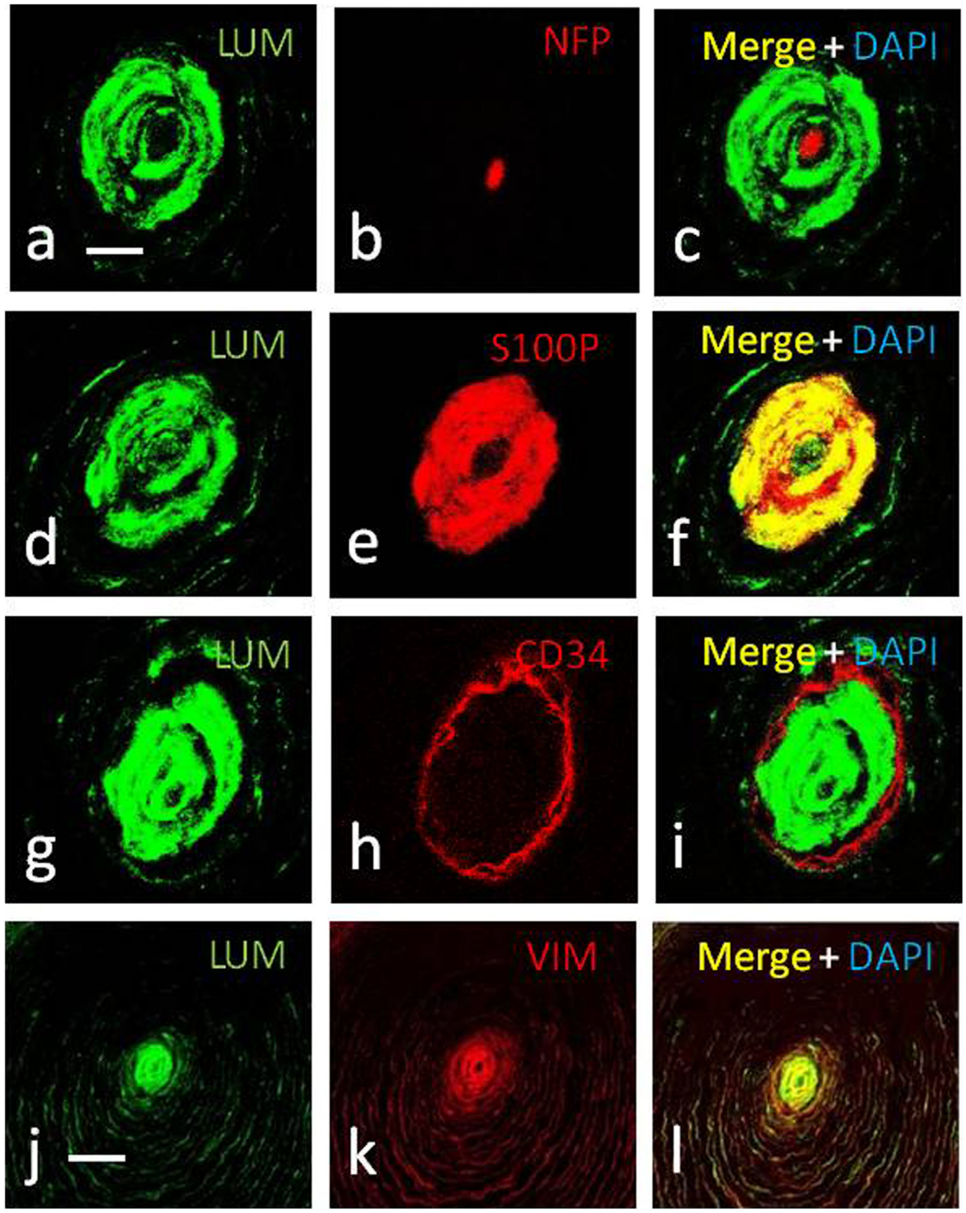

Fig. 5. Double immunofluorescence in Pacinian corpuscles for lumican (LUM) in green (a, d, g and j) and neurofilaments (NFP; b), S100 protein (S100P, e), CD34 (h) or vimentin (VIM, k) in red. LUM was localized in the inner core, co-localized with S100P (d–f), but not in the axon (a–c). It was also observed LUM immunoreactivity in the outer core and capsule associated to VIM (j–l), but not in the CD34-positive intermediate layer (g–i). Scale bar: 20 μm, a to i; 40 μm, j to l. Objective: 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm, a to i; 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm, j to l. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

membranes. In Pacinian corpuscles ECM fills the spaces between lamellae that form the inner core, intermediate layer and outer core-capsule (Ide, 1986). In some of these compartments ECM is organized as a BM (Malinovský, 1986; Vega et al., 1995; Dubový and Aldskogius, 1996; García-Piqueras et al., 2018). The current information about the composition of ECM in vertebrate Pacinian corpuscles regards to different types of collagen, laminin and two PGs (Malinovský, 1986; Vega et al., 1995, 1996; Sames et al., 2001). Thus, in the present study we have investigated the occurrence of other constituents of ECM, in particular class I (DCN, BGN and ASPN) and Class II (LUM, FMOD, OMD, KERA and PRELP) SLRPs.

A previous study carried out on Pacinian corpuscles from cat mesentery detected DCN and BGN (Sames et al., 2001), being found DCN in the outer core and BGN in the inner core. Present results in human digital Pacinian corpuscles matches those for DCN in the cat, but differs with respect to BGN distribution since they found it restricted to the inner core while we observed immunoreactivity in the inner core and also in the outer core. The role of these two molecules in Pacinian corpuscles is unknown. Evidence of the influence of DCN PGs in the cellular morphology *via* the stabilization of the cytoskeletal vimentin intermediate filaments has been recently reported (Jungmann et al., 2012) but the importance of this relationship in the Pacinian corpuscles outer core must be elucidated in additional studies.

As far as we know the occurrence and distribution of class II SLRPs in vertebrate Pacinian corpuscles was never investigated. Therefore, data provided in the present study cannot be contrasted with other studies.

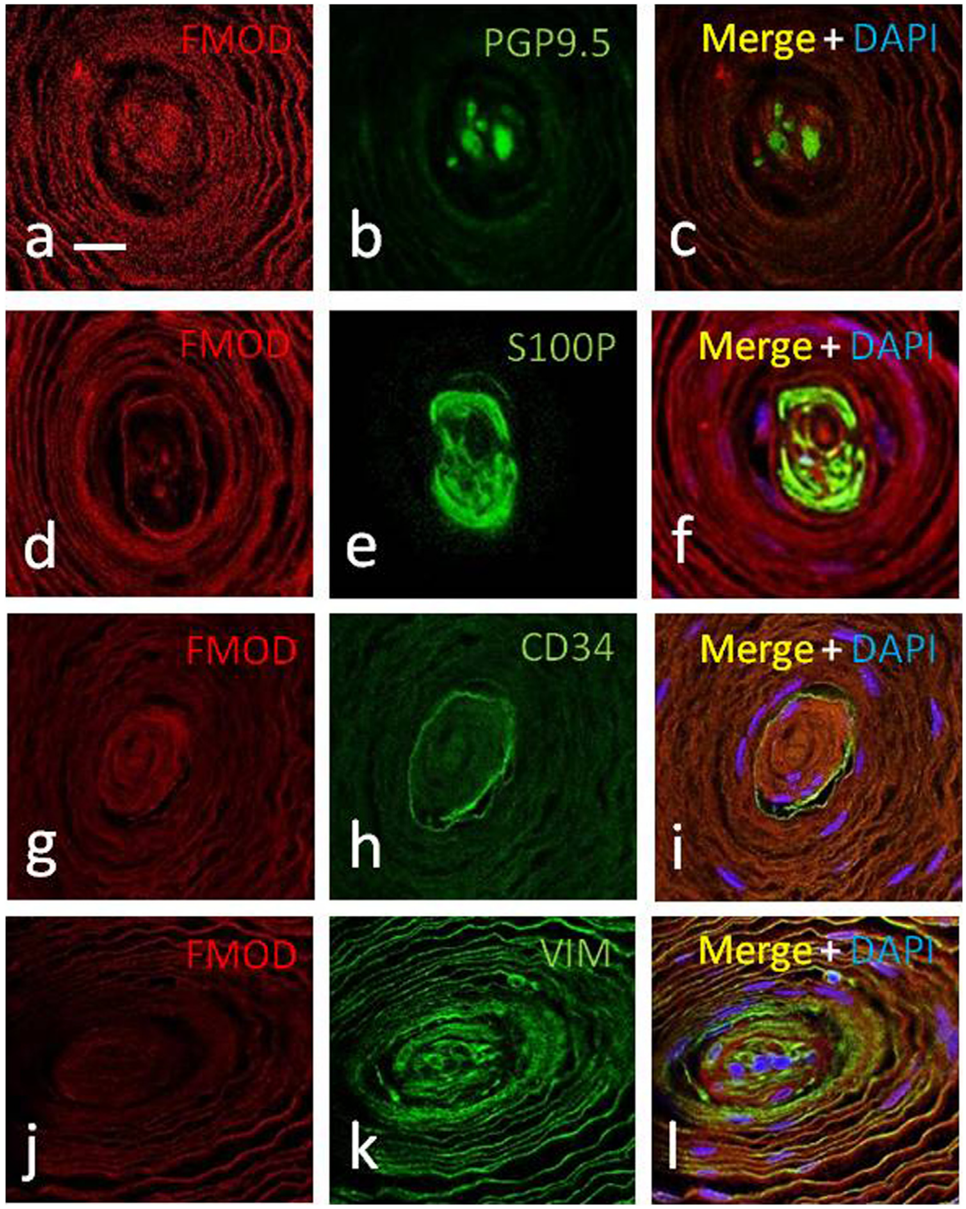

Fig. 6. Double detection of fibromodulin (FMOD) in red (a, d, g and j) and PGP9.5 (b), S100 protein (S100P, e), CD34 (h) or vimentin (VIM, k) in green by using confocal immunofluorescence in Pacinian corpuscles. Immunolabeling of FMOD was observed in the inner core but colocalizing irregularly with S100P (d–f). It was also found in the outer core and capsule, adjacent to VIM (j–l). On the other hand, FMOD was neither present in the axon (a–c), nor in the intermediate layer (g–i). Scale bar: 10 μm, a to l. Objective: 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm, a to l. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Biochemical and cell culture assays have shown that SLRPs modulate ECM organization by binding to various collagens (I, II, III, IV, V, VI, XII and XIV; Chen and Birk, 2013; Gubbiotti et al., 2016). In particular, LUM binds collagen II, BGN and FMOD interact with collagens II and VI, and DCN binds collagens II, IV, V and VI (Chen and Birk, 2013). The interlamellar spaces of Pacinian corpuscles contain different types of collagen (II, IV, V, VI; Tachibana et al., 1995; Vega et al., 1996; Pawson et al., 2000) and members of the class I and class II SLRPs proteins participate in the regulation of collagen fibril diameter and interfibrillar spacings (Chen and Birk, 2013; Tashima et al., 2015). SLRPs modulate ECM fibrillogenesis, organization and assembly (Kalamajski and Oldberg, 2010; Chen and Birk, 2013; Gubbiotti et al., 2016).

On the other hand, SLRPs regulate cell activity interacting with growth factor receptors (Hocking et al., 1998; Svensson et al., 1999; Ezura et al., 2000; Merline et al., 2009; Dellett et al., 2012; Iozzo and Schaefer, 2015; Neill et al., 2015). The relative proportion of collagen and SLRPs determine the mechanical properties of the tissues (Hansen et al., 2015). DCN and BGN bind to epidermal growth factor receptor (EGFR) and Erb4 (Schaefer and Iozzo, 2008; Schaefer and Schaefer, 2010; Iacob and Cs-Szabo, 2010) and the localization of those SLRPs in human Pacinian corpuscles observed here is similar to that reported for EGFR in human Pacinian corpuscles (Vega et al., 1994) or Erb4 in the murine Pacinian corpuscles (González-Martínez et al., 2007). Interestingly DCN interacts with EGFR to regulate collagen fibrillogenesis (Mohan et al., 2011).

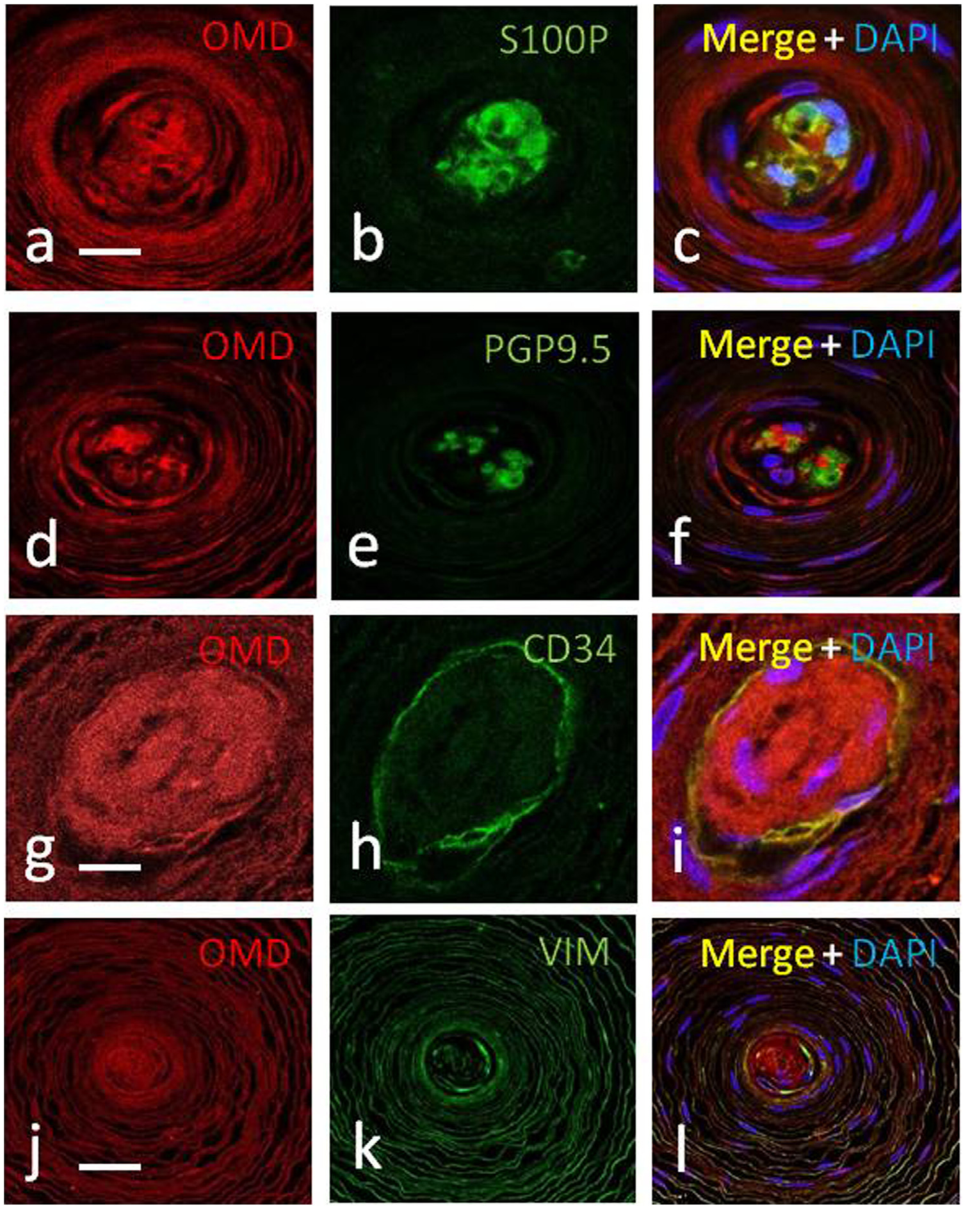

Fig. 7. Double immunofluorescence for simultaneous detection of osteoadherin (OMD) in red (a, d, g and j) with S100 protein (S100, b), PGP9.5 (e), CD34 (h) and vimentin (VIM, k) in green, in Pacinian corpuscles. OMD was expressed around the axon weakly (d–f), the inner core with an undefined pattern (a–c), the intermediate layer (g–i) and the outer core (j–l). VIM and OMD do not colocalized in the outer core, but both molecules were adjacent in every single lamella (j-l). Scale bar: 10 μm, a to f and g to i; 40 μm, j to l. Objective: 63X/1.40 oil, pinhole 1.37, XY resolution 139.4 nm and Z resolution 235.8 nm, a to I; 40X/1.25 oil, pinhole 1.00, XY resolution 156 nm and Z resolution 334 nm, j to l. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

SLRPs are expressed during neural development and in adult neural tissues (Le Goff and Bishop, 2007; Dellett et al., 2012), including Schwann cells (Hanemann et al., 1993), dorsal root ganglia (Wilda et al., 2000), and cat mesentery Pacinian corpuscles (Sames et al., 2001). PGs, including the molecules studied here, are involved in regenerative processes of the peripheral nervous system, both as neuritogenic molecules and as inhibitors of axonal regeneration (Braunewell et al., 1995; Oohira et al., 2000; Bradbury et al., 2002; Maeda et al., 2011; Soleman et al., 2013; Silver and Silver, 2014). DCN inhibits neurite outgrowth (Stichel et al., 1995; Davies et al., 2004), whereas BGN is a neurotrophic molecule (Junghans et al., 1995; Koops et al., 1996).

In conclusion, this study provides a comprehensive analysis of SLRPs localization in human cutaneous Pacinian corpuscle. Their complex expression patterns suggest a compartment-specific regulation of collagen fibrillogenesis and stromal matrix assembly and hydration. Considering the numerous SLRPs functions, they could be essential for determining Pacinian corpuscle elasticity, mechanical properties and structural integrity for the correct transmission of pressure resulting from indentation or stretch. The roles of all the SLRPs in the human Pacinian corpuscles await elucidation in the future.

Ethical statement

This study was performed in compliance with Spanish Law and the guidelines of the Helsinki Declaration II, and the study was

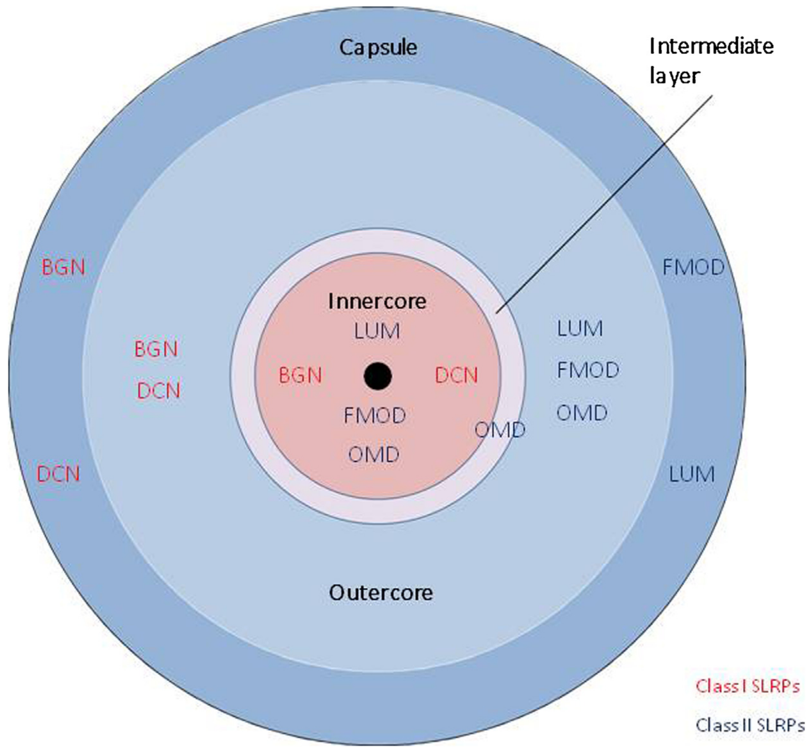

Fig. 8. Schematic representation of the transverse section of a Pacinian corpuscle showing the distribution of the analyzed SLRPs in the different corpuscular compartments, based on the results of double immunofluorescence.

approved by the Ethical Committee for Biomedical Research of the Principality of Asturias, Spain (Ref. 266/18).

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.aanat.2019.02.007.

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