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Expression of smoothelin and smooth muscle actin in the skin

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Summary. Introduction: Smoothelin is a cytoskeletal protein of differentiated smooth muscle cells with contractile capacity, distinguishing it from other smooth muscle proteins, such as smooth muscle actin (SMA).

Objective: To evaluate the expression of smoothelin and SMA in the skin in order to establish specific localizations of smoothelin in smooth muscle cells with high contractile capacity and in the epithelial component of cutaneous adnexal structures. Methods: Immunohistochemical analysis (smoothelin and SMA) was performed in 18 patients with normal skin.

Results: SMA was expressed by the vascular structures of superficial, deep, intermediate and adventitial plexuses, whereas smoothelin was specifically expressed in the cytoplasm of smooth muscle cells of the deepest vascular plexus and in no other plexus of the dermis. The hair erector muscle showed intense expression of smoothelin and SMA. Cells with nuclear expression of smoothelin and cytoplasmic expression of SMA were observed in the outer root sheath of the inferior portion of the hair follicles and intense cytoplasmic expression in cells of the dermal sheath to SMA.

Conclusions: We report the first study of smoothelin expression in normal skin, which differentiates the superficial vascular plexus from the deep. The deep plexus comprises vessels with high contractile capacity, which is important for understanding dermal hemodynamics in normal skin and pathological processes. We suggest that the function of smoothelin in the outer root sheath may be to enhance the function of SMA, which has been related to mechanical stress. Smoothelin has not been studied in cutaneous pathology; however we believe it may be a marker specific for the diagnosis of leiomyomas and leiomyosarcomas of the skin. Also, smoothelin could differentiate arteriovenous malformations of cavernous hemangioma of the skin.

Key words: Immunohistochemical, Smoothelin, Smooth muscle actin, Hair follicle, Vessels

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Introduction

Smoothelin has two tissue-specific isoforms: the short 59-kDa isoform (A), found in smooth muscle cells of the organs; and the long 110-kDa isoform (B), in smooth muscle cells of vascular structures (Krämer et al., 2001; Rensen et al., 2002). Smoothelin contains an actin-binding domain and is considered to be a cytoskeleton protein exclusively expressed by differentiated smooth muscle cells with contractile capacity (Krämer et al., 1999, 2001).

Different grades of differentiation of muscle cells in vessels are determinated by expression of phenotypic features such as smoothelin, myosin and actin of smooth muscle. Depending on the grade of differentiation of these cells, vessels have an important role in physiologic processes, such as thermoregulation with vaso-constriction and vasodilatation as a response to environmental temperature changes, and in physiopathologic processes such as atherogenesis and restenosis after angioplasty and other surgical techniques (Holifield et al., 1996; Hungerford and Little, 1999), as well as Raynaud phenomenon and erythromelalgia: two cutaneous microvascular disorders whose pathophysiological features are poorly understood (Greenstein et al., 1995; Davis et al., 2000).

Immunohistochemical studies have detected smoothelin expression in smooth muscle cells of the esophagus, stomach, gut, prostate, uterus, and bladder, and in leiomyoma, leiomyosarcomas, and gastrointestinal stromal tumors (Van der Loop et al., 1996; Council and Hameed, 2000; Niessen et al., 2005; Wedel et al., 2006; Amiot et al., 2009; Coco et al., 2009; Bovio et al., 2010). We have found only one published study on the cell localization of smoothelin expression, which evaluated its cytoplasmatic and nuclear expression in normal and tumor smooth muscle cells (Coco et al., 2009).

The objective of this study was to investigate the expression of smoothelin and smooth muscle actin (SMA) in the skin in order to establish specific localizations of smoothelin in smooth muscle cells with high contractile capacity and in the epithelial component of cutaneous adnexal structures.

Materials and methods

Patient samples

We studied 18 biopsies of normal skin that included the epidermis, dermis, and part of the hypodermis. They derived from flaps removed for grafting purposes from the limb or scalp of 10 females and 8 males aged between 25 and 48 yrs. Written informed consent was

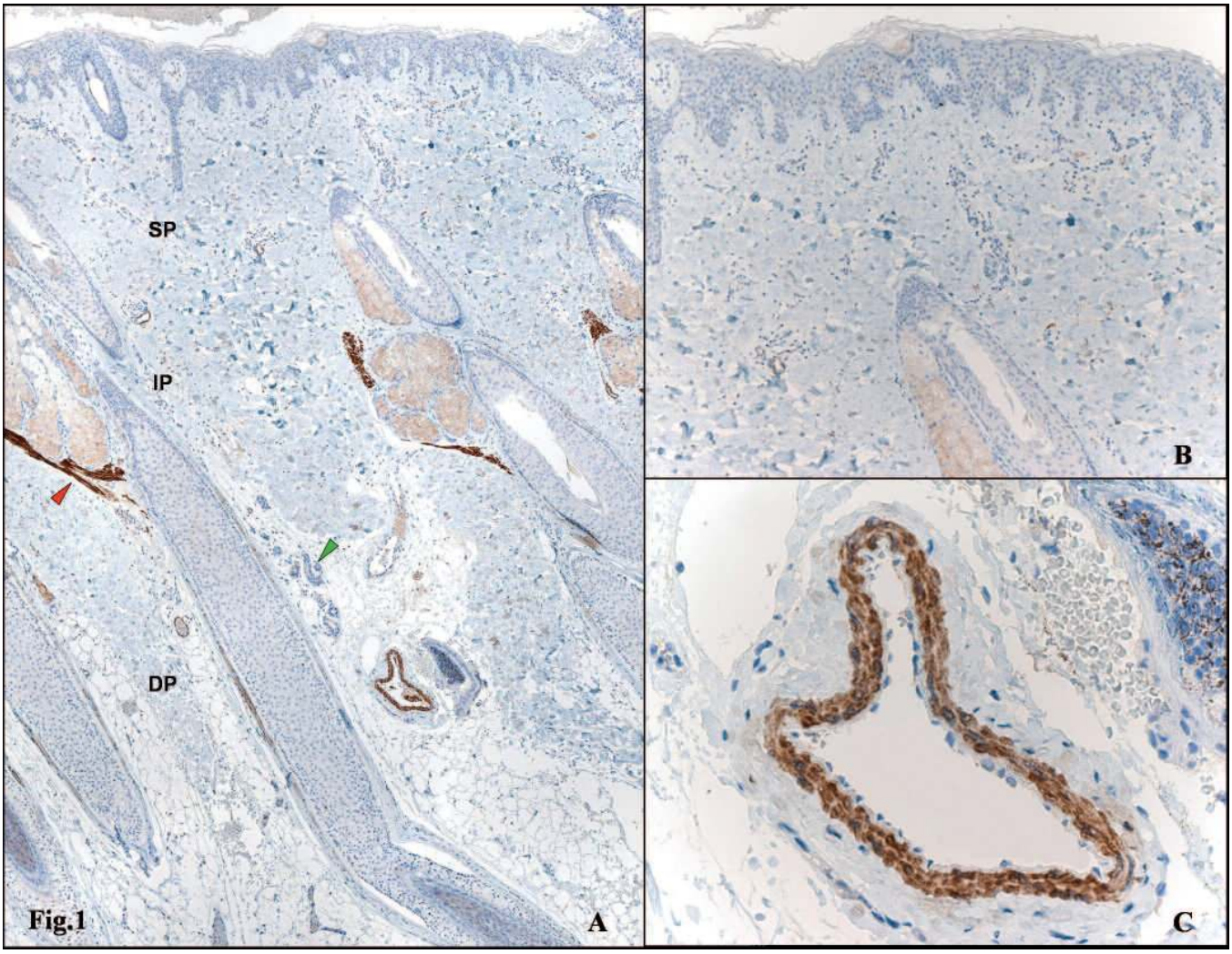

Fig. 1. A. Smoothelin expression is positive only in the vessel walls of deep plexus (DP), with no expression in the superficial plexus (SP). Some vessels of the intermediate plexus (IP) are also positive for smoothelin. Intense expression is found on hair erector muscle (Red arrow), however ecrine sweat glands shows no expression (Green arrow). **B**. On higher magnification, vessels of SP are negative for smoothelin. **C.** Vessel walls of DP are positive for smoothelin. A, x 100; B, x 200; C, x 400

obtained from all subjects.

Immunohistochemical analysis

Samples were fixed in 10% buffered formalin for 24 hrs and embedded in paraffin. Paraffin-embedded 4-μm sections were dewaxed, hydrated, and heat-treated at 95°C for 20 min in 1 mM EDTA buffer pH 8 for antigenic unmasking. Sections were incubated for 30 min at room temperature with smoothelin (prediluted monoclonal antibody, clone R4A) or SMA (prediluted clone 1A4) (Master Diagnóstica, Granada, Spain). The immunohistochemical study was done on an automatic immunostainer (Autostainer 480, LabVision Fremont, CA) by indirect polymer-peroxidase-based method followed by development with diaminobenzidine (Masvision, Master Diagnóstica). The intensity of cytoplasmic and/or nuclear expression was graded as weak, moderate, or strong.

Results

Smoothelin and SMA expression in the vascular structures of the dermis and dermal-hypodermal interface, comprising superficial, deep, intermediate (communicating vessels), and adventitial plexuses

All biopsies showed intense cytoplasmic expression of smoothelin in smooth muscle cells of arteries and moderate cytoplasmic expression in veins of the deep vascular plexus at the dermal-hypodermal interface. No expression was found in superficial or adventitial plexuses (Fig. 1).

All biopsies showed intense SMA expression in the vessel walls of deep, intermediate, superficial, and adventitial plexuses (Fig. 2).

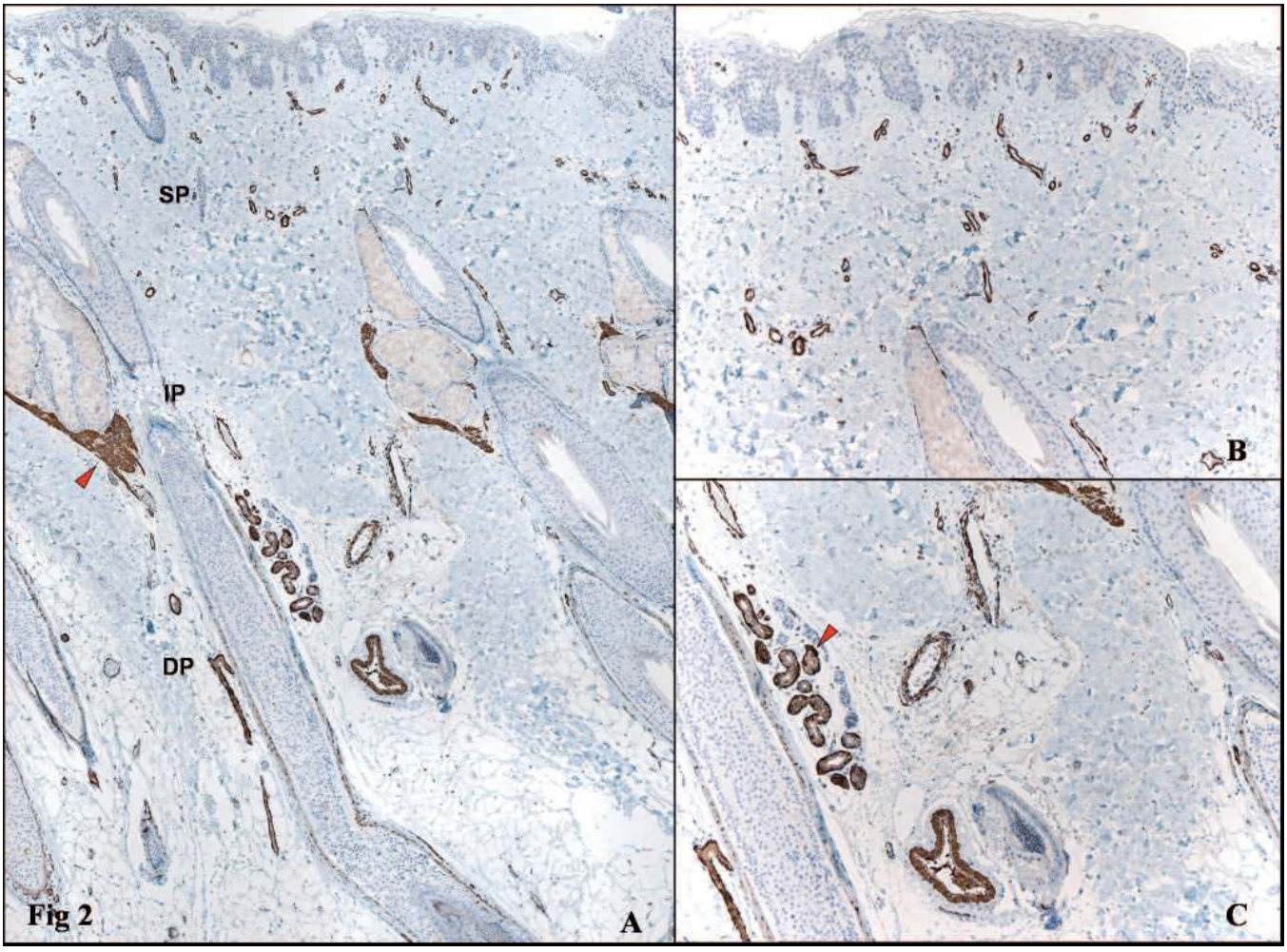

Fig. 2. A. Smooth muscle actin expression is noted in the vessel walls of deep plexus (DP), intermediate plexus (IP) and superficial plexus (SP). Intense expression is found on hair erector muscle (Arrows). **B.** On higher magnification, IP and SP show intense expression for smooth muscle actin. **C.** Vessels on the DP are positive for smooth muscle actin (Arrows). A, x 100; B, C, x 200

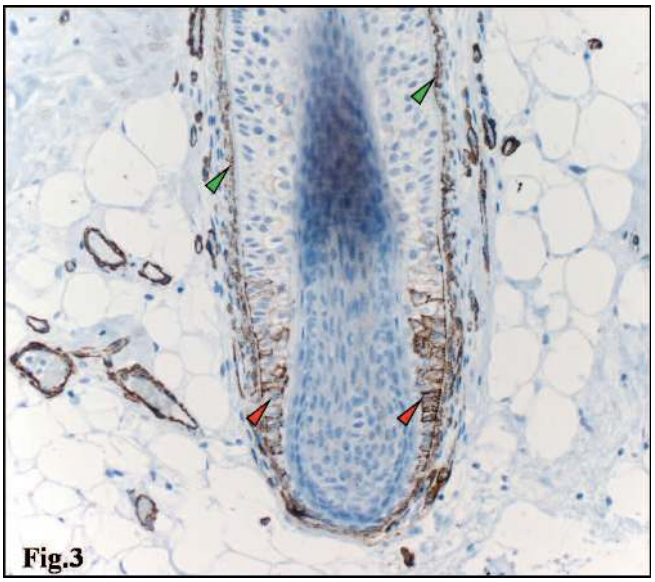

Fig. 3. Smooth muscle actin expression is positive in the dermal sheath (green arrows) and in the bottom area of the outer radicular sheath (red arrows). x 200

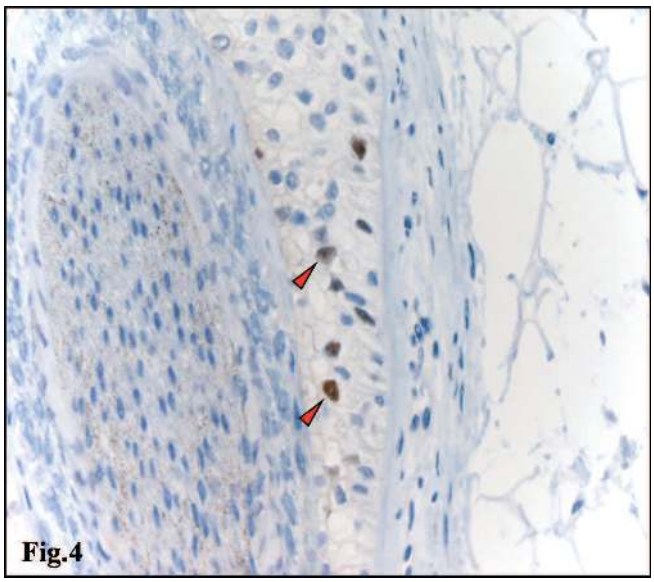

Fig. 4. Nuclear expression of smoothelin is noted in the cells of the outer radicular sheath (red arrows). x 400

Smoothelin and SMA expression in hair erector muscle

All biopsies showed intense cytoplasmic expression of smoothelin and SMA in smooth muscle cells of the hair erector muscle (Figs. 1A, 2A).

Smoothelin and SMA expression in hair follicle

All biopsies showed moderate cytoplasmic expression of SMA in cells of the inferior portion of the outer root sheath and intense cytoplasmic expression in cells of the dermal sheath (Fig. 3).

An intense nuclear expression of smoothelin was found in some cells of the inferior portion of the outer root sheath of all hair follicles in anagen (Fig. 4), while no expression was observed in the remaining layers of the hair structure.

Smoothelin and SMA expression in sweat glands (eccrine and apocrine) and sebaceous glands

No smoothelin expression was detected in sweat or sebaceous glands (Fig. 1A).

In all biopsies, an intense cytoplasmic expression of SMA was found in myoepithelial cells of the secretory portion of eccrine and apocrine glands (Fig. 2A), while no SMA expression was detected in the excretory portion or sebaceous glands.

No smoothelin or SMA expression was detected in other components, such as epidermis, dermal fibroblasts, hypodermal adipocytes, or nerve fibers.

Discussion

This study of normal skin biopsies found a higher expression of smoothelin in cells of the hair erector muscle than in muscle cells of deep plexus veins, reflecting the elevated contractile capacity of the hair erector muscle and suggesting that it may serve as a good positive control in smoothelin assays. An important finding was that SMA was identified all vascular structures of the dermal plexuses, but smoothelin expression allowed differentiation of the deep vascular plexus from the other structures, which may facilitate research into the homodynamic of the cutaneous structure normal and pathological dermatological processes, such as dermatitis, stasis, alterations caused by heat and cold which can present vasodilation and / or vasoconstriction. We also believe that smoothelin may help to understand the pathophysiological mechanisms involved in the different stages of rosacea. Thus the assessed value of smoothelin could help explain the vascular changes that occur in different processes in rosacea. Most blood vessels in the middle and superficial dermis have no substantial contractile capacity and showed no smoothelin expression.

Smoothelin expression in cutaneous vascular tumours has not been studied previously, although there is a work about the differential expression of smoothelin in brain vascular lesions, in which a positive expression in arteriovenous malformations and a negative expression in cavernous hemangioma were demonstrated (Uranishi et al., 2001). Since smoothelin is expressed in

well-differenciated muscle cells with contractile capacity from the deep vascular plexus, we think this expression can be used to distinguish arteriovenous malformations which consist of malformed vessels with thick walls, and muscle cells with contractile capacity which are smoothelin-positive. However, no well-differenciated muscle cells can be found in cavernous hemangioma, therefore these lesions are negative for smoothelin.

SMA expression in cells of the hair follicle dermal sheath cells has been implicated in contractile processes that may control hair follicle shortening (Thibaut et al., 2005), and play a role in curly hair follicle morphology. Cytoplasmic expression of SMA in cells of the outer root sheath has been related to stress mechanisms (Baltenneck et al., 2000; Thibaut et al., 2005). We report for the first time the nuclear expression of smoothelin in some cells of the outer root sheath. The only published report on the nuclear and cytoplasmic expression of smoothelin in (normal and tumor) smooth muscle cells offered no explanation of its nuclear expression (Coco et al., 2009). The nuclear expression of smoothelin in the hair outer root sheath can be helpful in the diagnosis of skin adnexal tumors whose origin is the outer root sheath, such as trichilemmoma and inverted follicular keratosis (Kurokawa et al., 2003).

Smoothelin isoforms are homologous with other cytoskeletal smooth muscle proteins and contain an actin-binding domain. Treatment of cells with α-amanitin induced the formation of an actin bundle network in the nucleus (Baltenneck et al., 2000; Zhu et al., 2004), and it is known that some types of stress (e.g., heart shock and dimethylsulfoxide treatment) can induce the nuclear translocation of actin in various eukaryotic cells (Iida et al., 1992; Wada et al., 1998). Hence, the nuclear expression of smoothelin may be attributable to a translocation mechanism, especially in tumor disease (e.g., leiomyosarcoma, lymphoma) (Abd et al., 2007; Coco et al., 2009). Based on these data, it can be proposed that smoothelin may act to enhance the role of SMA in the hair follicle.

In the present study, the nuclear expression of smoothelin and the cytoplasmic expression of SMA in epithelial cells of the hair follicle outer root sheath may be related to multipotential cell elements, which would explain the expression of the two muscle markers. It has long been known that the multipotent capacity of epithelial cells of the follicle outer root sheath affords them a critical role in the regeneration of damaged interfollicular epidermis (Jahoda et al., 1993), explaining the expression of a muscle marker in the outer epithelial sheath of the follicle. Likewise, epithelial-mesenchymal interaction is essential for hair follicle development (Tobin et al., 2003a,b). Various studies have reported the multipotent capacity of epithelial and mesenchymal cells, demonstrating that cells of the dermal papilla and outer hair follicle dermal sheath can differentiate into adipocytes and express bone differentiation markers such as alkaline phosphatase (Jahoda et al., 2003; McElwee et al., 2003). Multipotent stem cells have been described in the outer root sheath (Webb et al., 2004; Raposio et al., 2007), which under certain circumstances may give rise to epithelial cells that express smoothelin and SMA.

Smoothelin is expressed in non-cutaneous muscle tumors (Coco et al., 2009), and in our study the hair erector muscle and the walls of vessels from the deep vascular plexus are smoothelin-positive, therefore this expression can be implemented in the diagnosis of benign and malignant muscle tumors of the skin, e.g. leiomyoma and leiomyosarcomas.

This is the first report of the positive expression of smoothelin in the outer root sheath of the hair follicle and its possible functional link to SMA. Moreover, smoothelin allows the deep vascular plexus to be distinguished from remaining vascular structures of the dermis.

The authors have no conflict of interest to declare. All the authors approve the submission. All authors have participated sufficiently to take public responsibility for appropriate portions of the work.

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