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Eccrine Sweat Glands: Expression of Transforming Growth Factor-β and Bone Morphogenetic Protein Type I Receptors and Their Intracellular Signalling Smad Proteins

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The transforming growth factor-β superfamily is thought to be involved in the regulation and control of growth and differentiation. These growth factors signal through transmembrane serine/threonine kinase receptors. The activation of type I receptor kinase phosphorylates a family of intracellular signalling proteins called Smads. In the present study, we wanted to localize type I and type II receptors and Smad proteins in human eccrine sweat glands. Expression of transforming growth factor-β type I receptor was restricted to myoepithelial cells only, whereas bone morphogenetic protein receptor IA was found selectively within the duct epithelium of both the dermal portion and the acrosyringium. Bone morphogenetic protein receptor IB antibody gave a faint staining of secretory epithelium and myoepithelial cells. Smad proteins were identified in different parts of the eccrine sweat gland apparatus. In particular, Smad 1 and Smad 3 were localized within myoepithelial cells, whereas coils were stained weakly for Smad 1 and Smad 3. Smad 3 protein was also expressed by the duct epithelium. Smad 2, Smad 4, Smad 5, Smad 6 and Smad 7 were not identified in eccrine sweat gland epithelia. Our data provide evidence for transforming growth factor-β/bone morphogenetic protein signalling in the eccrine sweat gland and the selective expression of Smad proteins. Myoepithelial cells and duct cells have been identified as major targets of the transforming growth factor-β pathway. Possible functions are growth inhibition and control of myoepithelial differentiation. ***Key words: sweat glands; transforming growth factor β; Smad proteins; bone morphogenetic proteins.***

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The transforming growth factor-β (TGF-β) superfamily of polypeptide growth factors has been proposed to play a central role in embryogenesis, cellular growth and differentiation. These growth factors signal through heterodimeric complexes of type I and type II transmembrane serine/threonine kinase receptors. Activation of the receptor complex occurs when the type II receptor kinase transphosphorylates the GS domain of the type I receptor. This activates the type I receptor kinase, which transiently associates with and phosphorylates a family of intracellular signalling proteins called Smads (1, 2). Of these molecules, Smad 1 and Smad 5 transduce the signal of bone morphogenetic proteins (BMPs), and Smad 2 and Smad 3 the signals of activin and TGF-β (3 – 7). Smad 4 has been shown to form heterodimers with the other Smads and acts as a common transducer (8 – 11). Smad 1 and Smad 5 induce ventral mesoderm and Smad 2 and Smad 3 dorsal mesoderm, whereas Smad 4 acts synergistically with other pathway restricted Smads (1, 2). Smad 6 inhibits signalling, forming stable associations with type I receptors. It interferes with the phosphorylation of Smad 2 and the subsequent heterodimerization with Smad 4, but does not inhibit Smad 3. Smad 6 also inhibits the phosphorylation of Smad 1 that is induced by the BMP type IB receptor (12). Smad 7 is a TGF-β inducible antagonist of TGF-β signalling. Like Smad 6, Smad 7 associates in a stable way with the TGF-β receptor complex, inhibiting the phosphorylation of Smad 2 and Smad 3. TGF-β rapidly induces Smad 7 mRNA, suggesting that Smad 7 may participate in a negative feedback loop to control TGF-β responses (13, 14).

There are only a few published data about eccrine sweat glands and TGF-β. In human skin TGF-β2 was found in the upper portion of eccrine ducts and in eccrine poroma (15). Recently, mRNA of activin, another member of the TGF-β superfamily, has been detected in the footpad glands of rats (16).

In the present paper, TGF-β signalling of human sweat glands was investigated. We focused on Smad proteins and selected type I and type II receptors in order to investigate whether TGF-β signalling is of importance in these glands.

MATERIAL AND METHODS

Tissue specimen

A total of 48 formalin-fixed and paraffin-embedded tissue specimens were collected from the files of the Department of Dermatology, University of Jena, Germany. Sections 3 μm thick were cut and collected on silane-coated glass slides.

Preparation of antibodies

Specific antisera against BMPR-IA, BMPR-IB, BMPR-II, TGF-β-RI, and Smad 1, Smad 2, Smad 3, Smad 4, Smad 5, Smad 6 and Smad 7 were made against synthetic peptides corresponding to specific parts of the different proteins. Antisera were affinity purified using CNBr-activated Sepharose CL-4B (Pharmacia-LKB) columns with immobilized peptides as described previously (17).

Immunohistochemistry

Sections were deparaffinized, rehydrated in descending alcohol dilutions and immersed in phosphate-buffered saline (PBS). The slides were treated with 0.01% trypsin (T8003, Sigma) in PBS for staining with BMP-receptors and TGF-β-receptors. For immunohistochemistry with the anti-Smad-sera the slides were pre-treated in the microwave three times for 3 min in citrate buffer, pH 6.0. ABC peroxidase immunohistochemistry was performed essentially as described previously (17).

The antibodies were used in the following concentrations: BMPR-IA, BMPR-IB, BMPR-II, Smad 1 to Smad 5 and Smad 7 3 μg/ml; TGF-β-RI 1.5 μg/ml and Smad 6 2 μg/ml. Tissues were then incubated with biotinylated goat anti-rabbit IgG (Vector Laboratories, Burlin-

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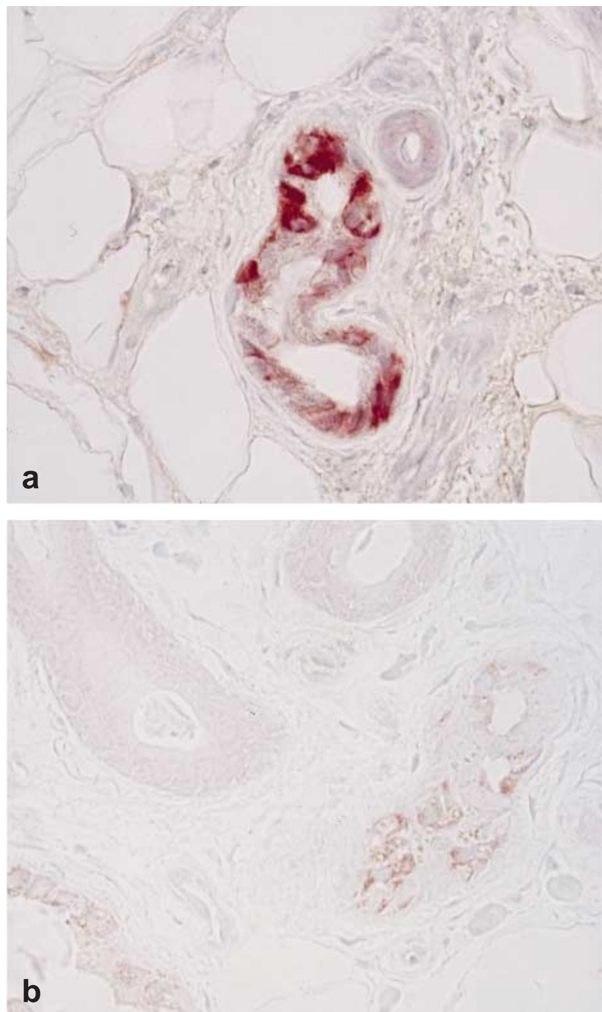

Fig. 1. Localization of transforming growth factor (TGF)-β and bone morphogenetic proein (BMP) receptors in eccrine sweat glands. (*a*) Strong TGF-β-RI expression in myoepithelial cells. (*b*) BMP receptor IB is weakly expressed by myoepithelial cells.

game CA, USA), followed by incubation with Vectastain ABC Elite complex (Vector Laboratories). The immunoreaction was visualized using 3-amino-9-ethylcarbazole (Merck) as a chromogen in the presence of 0.02% hydrogen peroxide, and finally counterstained with Mayer's hematoxilin and mounted in glycerol-gelatin. To exclude the non-specific reactions of secondary antibodies or ABC complexes, primary antibody solutions were replaced by 1% bovine serum albumin in PBS. The specificities of the antibodies were confirmed by blocking the immunohistochemical staining, after the antibodies had been pre-incubated with an excess molar ratio of the corresponding antigens.

RESULTS

The eccrine sweat gland is composed of epithelial and myoepithelial cells organized in 3 major segments: the secretory coil, the dermal duct and the epidermal duct or acrosyringium. Each part disclosed a distinct pattern of expression of TGF-β-superfamily type I receptors (transducer) and Smad proteins.

The secretory coils showed a strong reactivity for TGF-β type I receptor which was restricted to myoepithelial cells only (Fig. 1a). Antibodies against BMP receptor IB produced faint staining of secretory epithelium and myoepithelial cells (Fig. 1b; Table I). Smad proteins 1 and 3 were immunolocalized along the luminal surface of epithelial cells of the secretory coil. Smad 1 and Smad 5 were strongly expressed by myoepithelial cells. Staining was seen intracytoplasmatically (Fig. 2a).

Table I. *Expression of Smad proteins, bone morphogenetic-protein (BMP) receptor-IA/-IB, and transforming growth factor(TGF)-β type I receptor in eccrine sweat glands*

	SC	ME	DD	AC
Receptor proteins				
TGF-β type I receptor	–	+	–	–
BMP-receptor IA	–	–	+	+
BMP-receptor IB	(+)	(+)	–	–
Smad proteins				
Smad1	(+)	+	–	–
Smad2	–	–	–	–
Smad3	(+)	–	+	+
Smad4	–	–	–	–
Smad5	–	+	–	–
Smad6	–	–	–	–
Smad7	–	–	–	–

SC, secretory coil; ME, myoepithelial cells; DD, dermal duct; AC, acrosyringium.

The dermal duct is composed of 2 layers of epithelial cells but only the adluminal cells (inner layer) was labelled with antibodies against BMP receptor IA. The dermal duct was completely negative for BMP receptor IB and TGF-β type I receptor. Smad 3 was the only Smad protein which could be immunolocalized in inner dermal duct epithelium (Fig. 2b).

Staining of the acrosyringium provided results similar to the dermal duct, i.e. expression of BMP receptor IB and Smad 3. Antisera against Smad 2, Smad 4, Smad 6 and Smad 7 did not stain any part of the eccrine sweat gland (Table I).

DISCUSSION

The TGF-β signalling pathway has been related to embryogenesis, proliferation and differentiation control of a variety of different cells (1, 2). BMP 4 was found to be expressed in the dorsal neural tube and induce the expression of Msx2 in adjacent skin and mesenchyme. BMP 4 signalling may be an essential step for the establishment of the dorsal midline structures (18). BMP 2 and 4 play an active part in inhibition of cell growth in hair follicles and the onset of trichocyte-specific genes (19). In the developing epidermis, expression of BMP 6 coincides with the onset of stratification (20). TGF-β1 and TGF-β2 localized around hair follicles and skin glands during skin development seem to be involved in the regulation of morphogenesis and growth of skin appendages (21). The coordinated expression of several members of the TGF-β superfamily is required to control the progression of specific cell types through their differentiation pathway.

In the present study we investigated whether selected type I receptors and 7 different Smad proteins, responsible for intracellular signalling, were expressed by human sweat gland epithelium. Activation of type I receptors (transducer) triggers the assembly of heterodimeric complexes of the two types of Smads, the pathway-restricted and the common mediator

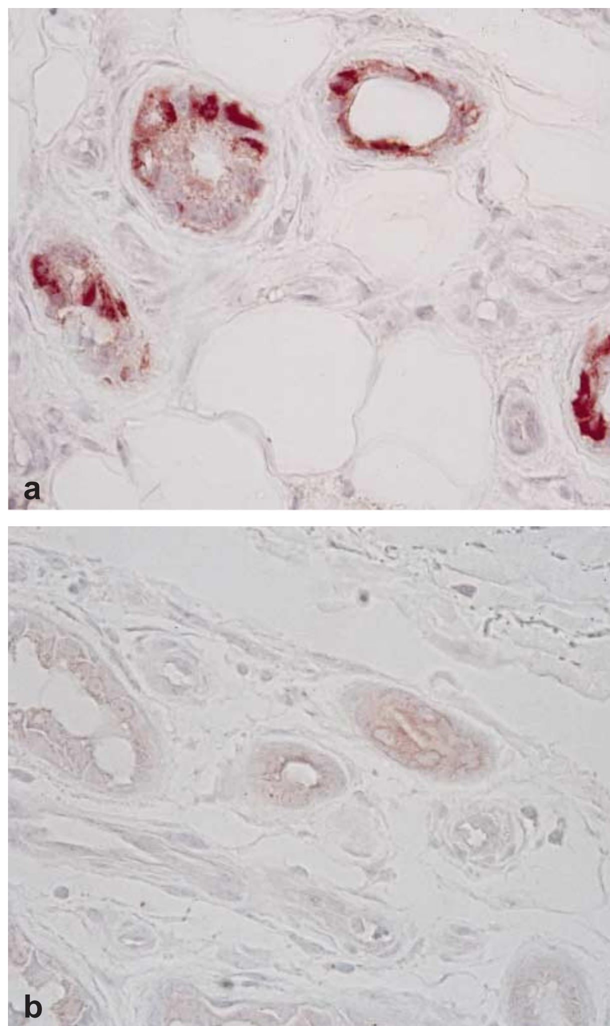

Fig. 2. Smad protein localization in eccrine sweat glands. (*a*) Strong expression of Smad 5 in myoepithelial cells and weak staining of secretory epithelium and (*b*) weak adluminal expression of Smad 3 in dermal ducts.

Smads, by phosphorylation of pathway-restricted Smads in their C-terminal SSXS motifs (1, 2).

We observed a restricted distribution of both receptor expression and Smad proteins within the major portion of the sweat gland apparatus. Myoepithelial cells are characterized by expression of TGF-β receptor type I and BMP receptor type IB, Smad 1 and Smad 3 proteins. Smad 1 is one of the transducers of BMP signalling, whereas Smad 3 transduces activin/TGF-β signals (15, 22–24). The pathway TGF-β→TGF-β receptor type II→TGF-β receptor type I→Smad 3 is known for growth inhibitory and extracellular matrix effects. During embryogenesis it is also involved in the induction of the dorsal mesoderm (1, 2). Myoepithelial cells seem to be the only cell type of eccrine glands to be regulated by BMP. BMP has been detected in the epidermis and the dermis, but not in eccrine sweat glands (19, 25). Activin mRNA was demonstrated within the duct epithelium, but not in secretory coils. Activins have been identified as possible cholinergic differentiation factors that are known to alter the phenotype of sympathetic neurons that innervate the sweat gland *in vivo*. TGF-β2 was detected only in the upper portion of duct epithelium (17, 26). Therefore it is reasonable to conclude that dermal BMP controls myoepithelial differentiation. In transformed or modified myoepithelial cells, however, as in mixed skin tumours, BMP expression has been observed (25).

The secretory epithelium expressed neither TGF-β type I receptor nor BMP receptor IA, but stained weakly for BMP receptor IB. Secretory coil epithelium disclosed only a faint staining for Smad proteins Smad 1 and 3, in particular on the luminal site. Since the BMP 2/4 signalling via BMP receptor type II and type IB, which stimulates Smad 1 intracellulary has been linked to neural differentiation and ventral mesoderm induction, the immunolocalization of BMP receptor IB and Smad 1 in secretory coils may be in favour of the hypothesis of a mesectodermal origin of eccrine glands (27, 28).

The dermal duct and the acrosyringium stained for BMP receptor IA and Smad 3. Both activin and TGF-β ligands have been demonstrated in the duct epithelium and Smad 3 is their major transducer. Epidermal BMP 2 and BMP 4 may stimulate BMP receptor IA by binding to activin receptors and inducing growth inhibition by Smad 3 (1, 2). Interestingly, none of the inhibitory Smads, such as Smad 6 or Smad 7, have been detected in the eccrine gland.

There are few data on this pathway in other skin appendages. TGF-β1 has been demonstrated in murine hair follicles together with Tβ RI and Tβ RII. The latent TGF binding protein (LTBP) was demonstrated in sebaceous glands (29, 30). In human skin TGF-β2 but not TGF-β1 is expressed by epidermal keratinocytes, hair follicles and sebaceous glands, but expression of Tβ RI and Tβ RII is weak. In basal cell carcinomas TGF-β2 may be absent (15).

Our results suggest that TGF-β signalling is of importance for human eccrine sweat glands. The type of expression and distribution, however, is unique among skin appendages. Whereas the dermal duct and the acrosyringeum show close similarities with respect to the expression of TGF-β superfamily receptors and Smad proteins, the secretory coil including myoepithelial cells are different. Myoepithelial cells and duct cells have been identified as major targets of the TGF-β pathway. But myoepithelial cells seem to be regulated exclusively by BMP and TGF-β. The BMP regulation of myoepithelial cells could be responsible for the change of their phenotype in hidradenomas and mixed tumours of skin to chondroid cells (28). Our findings also support the hypothesis of a mesectodermal origin of eccrine sweat glands in contrast to hair follicles or sebaceous glands (cf. 27, 28).

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