Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1758 (2006) 1034–1042
BIOCHIMICA ET BIOPHYSICA ACTA
BBA
www.elsevier.com/locate/bbamem

Expression and function of aquaporins in human skin: Is aquaporin-3 just a glycerol transporter?

M. Boury-Jamot [a], R. Sougrat [a,1], M. Tailhardat [b], B. Le Varlet [b], F. Bonté [b], M. Dumas [b], J.-M. Verbavatz [a,*]

[a] *Service de Biophysique des fonctions membranaires, DBJC/SBFM, Bâtiment 532, CEA/Saclay, F-91191 Gif-sur-Yvette, Cedex, France*
[b] *LVMH Recherche, F-45804 St Jean de Braye, France*

Received 22 December 2005; received in revised form 17 May 2006; accepted 6 June 2006
Available online 18 June 2006

Abstract

The aquaporins (AQPs) are a family of transmembrane proteins forming water channels. In mammals, water transport through AQPs is important in kidney and other tissues involved in water transport. Some AQPs (aquaglyceroporins) also exhibit glycerol and urea permeability. Skin is the limiting tissue of the body and within skin, the stratum corneum (SC) of the epidermis is the limiting barrier to water loss by evaporation. The aquaglyceroporin AQP3 is abundantly expressed in keratinocytes of mammalian skin epidermis. Mice lacking AQP3 have dry skin and reduced SC hydration. Interestingly, however, results suggested that impaired glycerol, rather than water transport was responsible for this phenotype. In the present work, we examined the overall expression of AQPs in cells from human skin and we reviewed data on the functional role of AQPs in skin, particularly in the epidermis. By RT-PCR on primary cell cultures, we found that up to 6 different AQPs (AQP1, 3, 5, 7, 9 and 10) may be selectively expressed in various cells from human skin. AQP1, 5 are strictly water channels. But in keratinocytes, the major cell type of the epidermis, only the aquaglyceroporins AQP3, 10 were found. To understand the role of aquaglyceroporins in skin, we examined the relevance to human skin of the conclusion, from studies on mice, that skin AQP3 is only important for glycerol transport. In particular, we find a correlation between the absence of AQP3 and intercellular edema in the epidermis in two different experimental models: eczema and hyperplastic epidermis. In conclusion, we suggest that in addition to glycerol, AQP3 may be important for water transport and hydration in human skin epidermis.

Keywords: Aquaporin; AQP3; Human skin; Epidermis; Glycerol transport; Water channel; Keratinocyte; Aquaglyceroporin

1. Introduction

1.1. Aquaporins

The aquaporins (AQPs) are a family of membrane proteins that form water channels across cell membranes. Some aquaporins can also transport small solutes like glycerol or urea. Several hundred AQPs have been identified in various organisms including bacteria, yeast, insects, plants and animals. There are 13 aquaporins in mammals (named AQP0 to AQP12). AQP1, 2, 4, 5 and 8 appear to function as selective water channels, while AQP3, 7, 9 and 10 are also permeable to small solutes including glycerol. The tissue distribution, cellular localization, regulation, structure and function of mammalian AQPs have been extensively studied. However, the functional importance of AQPs is best understood in kidney, where AQP1, 2, 3, 4 are involved in water reabsorption and urine concentration, whereas AQP7 is involved in the transport of glycerol. The physiological role of some AQPs in humans has been confirmed by the phenotype observed in individuals where one AQP, was mutated, like AQP2 in nephrogenic diabetes insipidus. Unfortunately, no specific inhibitor or activator of AQP function is available. Thus, most data on the physiological role of AQPs come from transgenic mice.

1.2. Mammalian skin

Skin is composed of three regions (Fig. 1): the deepest region is the hypodermis, containing adipocytes. Above the hypodermis

* Corresponding author. Tel.: +33 1 6908 2665; fax: +33 1 6908 8029.
E-mail address: jean-marc.verbavatz@cea.fr (J.-M. Verbavatz).
[1] Current address: Cell Biology and Metabolism, NICHD, NIH, Bethesda, MD, USA.

0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.06.013

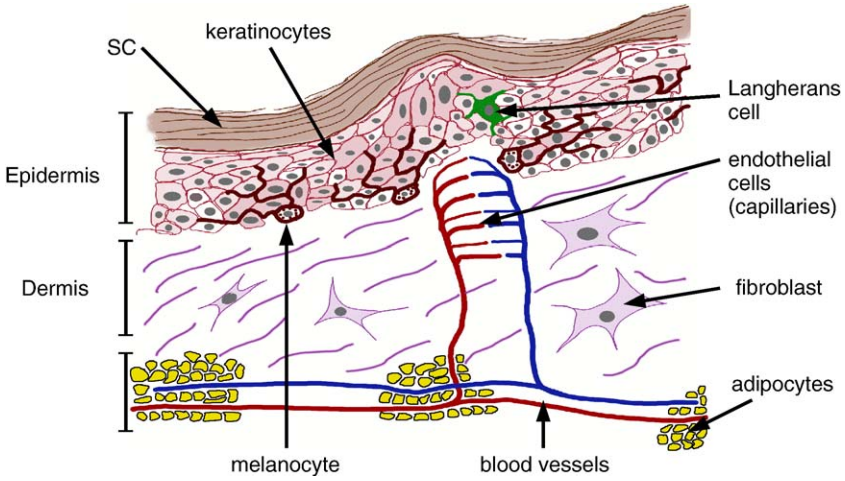

Fig. 1. Schematic structure of human skin. Skin is divided in three regions: epidermis (top, the apex and permeability barrier of skin) is primarily composed of keratinocytes which proliferate at the base, differentiate, migrate towards the surface and terminally differentiate into corneocytes in the stratum corneum (SC). Melanocytes (in the basal layer of the epidermis), which produce melanine, and Langerhans cells are also localized in the epidermis. The dermis is a loose connective tissue containing collagen, elastin, some fibroblasts and small blood vessels. Adipocytes are localized deeper in skin hypodermis.

is the dermis, which is of a complex matrix collagen and elastin with a network of capillaries and a few fibroblasts. The most superficial region of skin is the epidermis, which is supported by the dermis. The epidermis is an epithelium which is permanently renewed: keratinocytes proliferate in the basal layer of the epidermis and they differentiate in the stratum spinosum and stratum granulosum as they stratify and migrate towards skin surface. Finally, The stratum corneum (SC) is composed of terminally differentiated keratinocytes; corneocytes. Despite keratinocytes are the principal cell type of the epidermis (~95% of cells), melanocytes and Langerhans cells are also located in skin epidermis. The primary barrier of the epidermis against xenobiotics, loss of body fluids and water loss are the lipid matrix and corneocytes of the SC. The water contents of skin is remarkably high in basal layers of the epidermis (~75% water) and drops sharply in the stratum corneum which contains only 10–15% water [26]. Proper hydration of superficial SC, with highly organized lipid/water lamellar structures between corneocytes is important to maintain the permeability barrier of the skin, but lower SC is mostly unable to absorb water [5]. The discontinuity in water contents which occurs between the stratum granulosum and the SC [26] is essential to maintain the structure of the epidermis: corneocytes constitute the low water content permeability barrier, but they originate from keratinocytes which need water to proliferate and differentiate.

1.3. Skin aquaporins

The presence of water channels in amphibian skin epidermis has been known for a long time [3]. In contrast, aquaporins were not expected in mammalian epidermis which is impermeable to water. Yet, AQP3 was detected in keratinocytes of rat epidermis shortly after it was cloned [8]. Later, we demonstrated *in vitro* and *in vivo* that AQP3 was expressed in the plasma membrane of human keratinocytes and functional as a pH-sensitive water channel [24]. By RT-PCR analysis, only AQP3 was found in the epidermis in mice [18], but AQP1 was detected in endothelial cells of the human dermis [19]. In addition, AQP5 is expressed in sweat glands where some authors have concluded that AQP5 was not physiologically involved [23], whereas others have argued that AQP5 was essential for sweat secretion [21]. Finally, AQP7 in adipocytes has been shown to play an important role in glycerol transport [13,15]. Thus in skin, the AQP7 protein can be expected in adipocytes of the hypodermis.

1.4. Functions of aquaporins in skin

Among skin AQPs listed above, only AQP3 in the epidermis and AQP5 in sweat glands are strictly relevant to skin physiology, because AQP1 and AQP7 are found respectively in endothelial cells [6,19,22] and adipocytes [13,15] throughout the body. AQP5 is believed to be a specific water channel [21,23]. Thus if AQP5 plays a physiological role in sweat secretion, one has to assume that it is water transport. In contrast AQP3 is an aquaglyceroporin, permeable both to water and glycerol. Therefore, studies were done to answer the question: is skin AQP3 important, and is it primarily involved in water, or in glycerol transport? In a first study, we showed that AQP3 was a functional water channel in human skin [24]. In mice, it was shown that AQP3 deletion results in normal skin structure, but decreased SC hydration (dry skin) and decreased water and glycerol epidermis permeabilities [18]. Later, reduced skin elasticity, delayed barrier recovery and reduced glycerol content in the SC were demonstrated [10]. Finally, glycerol replacement (i.e. glycerol administered topically, orally or by intra-peritoneal injection) was found to correct hydration, elasticity and barrier function [11]. The latter observation suggested that defective glycerol transport is sufficient to account for the skin phenotype of AQP3 deletion. Thus, glycerol transport, not water transport, appeared to be the function of AQP3 in mouse skin epidermis [12].

1.5. Aquaporins in human skin

In the present work, our goal was to provide a comprehensive pattern of AQP expression in human skin and to examine whether conclusions on AQP function in skin, primarily obtained from mice, were applicable to human skin. Because skin is composed of several different cell types, we investigated the expression of human AQPs mRNA for AQP0 to AQP10, in primary cultures of various normal cell types from human skin by RT-PCR. Cells tested for AQP expression included human keratinocytes, melanocytes, dermal fibroblasts, dermal microvascular endothelial cells, white preadipocytes, differentiated preadipocytes, monocytes and monocyte-derived dendritic cells. Sweat and sebaceous gland cells were not tested. In addition to AQPs expected from previous studies in humans or other mammals (including AQP3 in keratinocytes), we found AQP10 in keratinocytes, AQP1 in melanocytes and AQP9 in preadipocytes. Thus, up to 6 AQPs (AQP1, 3, 5, 7, 9, 10) may be expressed in human skin. Specific inhibitors, or natural mutations of these proteins in humans being currently unavailable, we then examined the putative physiological role of human skin AQPs based on previously published data and new observations.

2. Materials and methods

2.1. Tissues and cells

Primary cultures of various human skin cell types were performed as recommended by manufacturer from cells and specific media purchased from PromoCell (Heidelberg, Germany). Cells used in this study were normal human epidermal melanocytes from foreskin (NHEM.f-c), normal human white preadipocytes (HWP-c), normal human dermal fibroblasts (NHDF-c), normal human dermal microvascular endothelial cells (HDMEC-c), normal human epidermal keratinocytes from foreskin (NHEK.f-c). To obtain differentiated preadipocytes, white preadipocytes were first grown in growth medium until confluence, followed by preadipocyte differentiation medium (PromoCell, Heidelberg Germany) for 3 days and adipocyte nutrition medium for 2 weeks. Monocyte-derived dendritic cells (MDDCs) were generated from peripheral blood monocytes supplied by EFS Poitiers as buffy coat. Monocytes were purified with negative isolation kit using magnetic microbeads, cultured for 6 days in GM-CSF and IL-4 supplemented RPMI 1640 medium, resulting in the development of immature MDDC, as confirmed by FACS phenotypic analysis of maturation markers specific for dendritic cells. Normal skin biopsies were obtained from donors after facial or abdominal plastic surgery. Sections of biopsies from patients suffering from eczema were provided by the dermatology department of the Lyon-Sud hospital center.

2.2. RT-PCR analysis

Total RNA was extracted in triplicates from confluent 75 cm^2 cell culture flasks using RNeasy kits (Qiagen, Courtaboeuf, France). RT-PCR was performed with SuperScript One-Step RP-PCR with Platinum Taq (Invitrogen). Each reaction contained 100 ng total RNA (1 μl) for a total reaction volume of 50 μl. RT was done at 50 °C for 30 min and followed by 38 cycles of PCR with 52 °C annealing temperature. Amplification products were detected from 10 μl of reaction product stained by ethidium bromide in 2% agarose gel. The identity of amplification products was confirmed by their size and by the analysis of digestion profiles by specific restriction enzymes. The sequence of primers used for PCR amplification of AQP fragments and the expected length of amplicons were as follows:

AQP0 (length=652 bp): Sense: 5′-TGGCTATGGCATTTGGCTTG-3′
Antisense: 5′-TGGGTGTTCAGTTCAACAGGTTC-3′

AQP1 (length=711 bp): Sense: 5′-CTTTGTCTTCATCAGCATCGGTTC-3′
Antisense: 5′-ATGTCGTCGGCATCCAGGTCATAC-3′
AQP2 (length=534 bp): Sense: 5′-GCGTTTGGCTTGGGTATTGG-3′
Antisense: 5′-AAACAGCACGTAGTTGTAGAGGAGG-3′
AQP3 (length=781 bp): Sense: 5′-ACCCTCATCCTGGTGATGTTTG-3′
Antisense: 5′-TCTGCTCCTTGTGCTTCACAT-3′
AQP4 (length=839 bp): Sense: 5′-TTTCAAAGGGGTCTGGACTCAAG-3′
Antisense: 5′-CAACGTCAATCACATGCACCAC-3′
AQP5 (length=476 bp): Sense: 5′-CTCTTGGTGGGCAACCAGATC-3′
Antisense: 5′-TCACTCAGGCTCAGGGAGTTGG-3′
AQP6 (length=488 bp-spliced form, 894-unspliced form):
Sense: 5′-GCACTTCCCTCCGTGCTACAG-3′
Antisense: 5′-TGGACTGTGAACTTCCCAATGATG-3′
AQP7 (length=720 bp): Sense: 5′-GGGAGCTACCTTGGTGTCAACTT-3′
Antisense: 5′-CATCTTGGGCAATACGGTTATCC-3′
AQP8 (length=744 bp): Sense: 5′-GCCATGTGTGAGCCTGAATTTG-3′
Antisense: 5′-CTTCCCATCTCCAATGAAGCAC-3′
AQP9 (length=664 bp): Sense: 5′-ACGTTTTGGAGGGGTCATCAC-3′
Antisense: 5′-CAGGCTCTGGATGGTGGATTTC-3′
AQP10 (length=651 bp): Sense: 5′-ATAGCCATCTACGTGGGTGGTAAC-3′
Antisense: 5′-TTTGTGTTGAGCAGACACCAGATC-3′

2.3. Oocyte swelling assay

Osmotic water permeability of AQP3 was measured with a *Xenopus* oocyte swelling assay: 10 ng AQP3 mRNA was injected into oocytes. The swelling assay was performed 72 h later in five-fold diluted Barth's buffer at 22 °C. The osmotic permeability was calculated from the initial slope of swelling as reported previously [1]. For glycerol permeability measurements, the swelling was performed in an iso-osmotic solution (200 mosM final) containing 150 mM Glycerol in diluted Barth's buffer. The increase in oocyte volume then corresponds to isotonic glycerol uptake, which is accompanied by water as previously reported [1].

2.4. Immunofluorescence

For indirect immunofluorescence, tissues were fixed in 4% paraformaldehyde, and washed in PBS. 5 μm-thick cryosections were collected on glass slides. Slides were preincubated in PBS containing 1% BSA, then incubated in a 1:500 dilution of polyclonal rabbit serum containing antibodies against the C terminus sequence of AQP3 [24]. Following washes in PBS, slides were incubated with a secondary FITC-conjugated anti rabbit antibody and washed in PBS again. Finally, slides were counter-stained with Evans blue (red fluorescence), mounted in 50% glycerol containing 2% n-propyl-gallate and observed at the fluorescence microscope (Olympus van OX AH2).

2.5. Electron microscopy

Tissue samples were fixed in 4% paraformaldehyde and 0.1% glutaraldehyde and washed several times in PBS. They were postfixed in a 1:1 mixture of 2% osmium tetroxide and 3% potassium ferrocyanide. Following embedding in epon, 90 nm sections were cut, stained for 2 min in lead citrate and photographed at the electron microscope.

3. Results and discussion

3.1. Aquaporin expression in human skin

The expression pattern of AQP mRNA in primary cultures of human skin cells was investigated by RT-PCR from total RNA extracted from cell cultures with primers specific for human AQP0 to AQP10. For each cell type, RT-PCR was performed in triplicate RNA samples at least twice per sample. Fig. 2 shows representative results from skin cells isolated from the hypodermis and the dermis. AQP9 was detected in preadipocytes (Fig.

2a), but differentiated preadipocytes showed AQP7 instead (Fig. 2b). Dermal fibroblasts (Fig. 2c) and dermal microvascular endothelial cells (Fig. 2d) showed AQP1 mRNA. Most of these results are consistent with previous observations in other species and/or other tissues: Leitch et al. [16] have reported AQP1 in fibroblasts from mice, AQP1 in endothelial cells has previously been reported [6,19,22], AQP7 is an important glycerol transporter of adipocytes [13,15]. AQP9 in preadipocytes is a novel finding. Fig. 3 shows representative experiments in cells from the epidermis. As expected, keratinocytes, which are by far the principal cell type of the epidermis showed a strong signal for AQP3, but also a signal for AQP10 (Fig. 3a). Human melanocytes showed a signal for AQP1 (Fig. 3b), monocytes showed AQP9 and AQP10 expression (Fig. 3c), and monocyte-derived dendritic cells (MDDCs), used as a model of Langerhans cells of the epidermis showed AQP3 and AQP9 (Fig. 3d). To our knowledge, there have been no previous report of AQP1 expression in melanocytes. Despite we did not find AQP7 in MDDCs, the later finding is rather consistent with previously published data. Indeed, de Baey and Lanzavecchia [4] reported that AQP9 was expressed in all monocytes and not influenced by maturation, but AQP3 and AQP7 were selectively expressed in immature dendritic cells cultured with a cytokine combination that promotes generation of Langerhans cells. Thus, our findings suggest that up to 4 different AQPs (AQP1, 3, 9, 10) may selectively be expressed in human skin epidermis. It is interesting to note that AQP3, 9, 10, which are all aquaglyceroporins, were almost always co-expressed with an other aquaglyceroporin. AQP9 was often found in precursors of differentiated cells (i.e. preadipocytes and monocytes). AQP9 was also found by others during differentiation in human keratinocytes [25], but we were unable to reproduce this result.

3.2. Roles of aquaporins in human skin

In the dermis, we found AQP1, which is a selective water channel, in fibroblasts and endothelial cells. The dermis (Fig. 1) is a connective tissue rich in collagen, elastin and proteoglycans which is normally highly hydrated and where water can easily move by diffusion. Leitch et al. [4] reported that AQP1 was up-regulated in fibroblasts during hypertonic stress. They concluded that AQP1 regulation in fibroblasts was physiologically relevant in response to interstitial hyperosmolarity in lung airways and in kidney, but no direct evidence that this is important is available to our knowledge. In contrast, the role of AQP1 as a specific water channel in non-fenestrated endothelial cells is well established: for instance in the peritoneum, where water movement have been fully modelized, it has been demonstrated that water exchange through AQP1 in endothelial cells permits rapid osmotic equilibration between blood and the peritoneum [6]. Likewise, it is now well established that AQP7 in adipocytes is important for glycerol transport. Indeed, AQP7 deletion results in adipocyte hypertrophy, obesity and insulin resistance due to glycerol accumulation and enhanced triglyceride synthesis in adipocytes [13,15].

In the epidermis (Fig. 1), only AQP3 had been detected previously by RT-PCR [18]. Here, In human epidermis cells, we found AQP1, AQP3, AQP9 and AQP10 mRNA. The contribution of melanocytes to skin water homeostasis and to hydration of the epidermis cannot be very significant in view of the small proportion of melanocytes in skin epidermis (~3%). Like in fibroblasts however, AQP1 expression in melanocytes may be important for their water homeostasis, possibly in response to hypertonic stress. The AQP1 protein expression and localization in melanocytes is currently being investigated in our laboratory.

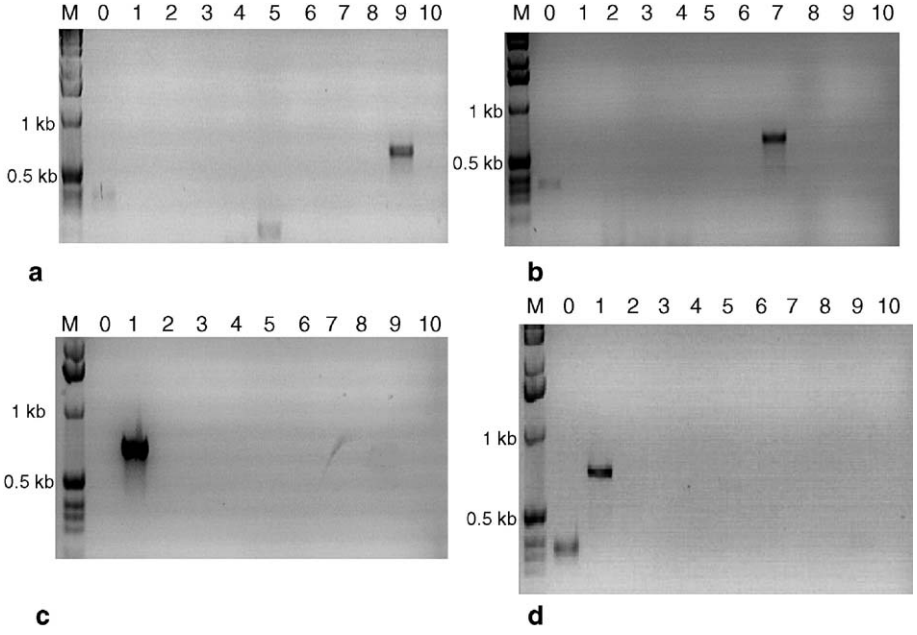

Fig. 2. RT-PCR analysis of aquaporin expression in primary cultures of cells from human dermis and hypodermis. M: size marker. 0–10: DNA transcribed from cell culture mRNA was amplified by PCR with primers specific for human AQP0 to AQP10. (a) In preadipocytes, only AQP9 was detected (amplification product: 664 bp). (b) Differentiated preadipocytes showed AQP7 instead (720 bp). AQP1 was detected in dermal fibroblasts (c) and dermal endothelial cells (d) (711 bp).

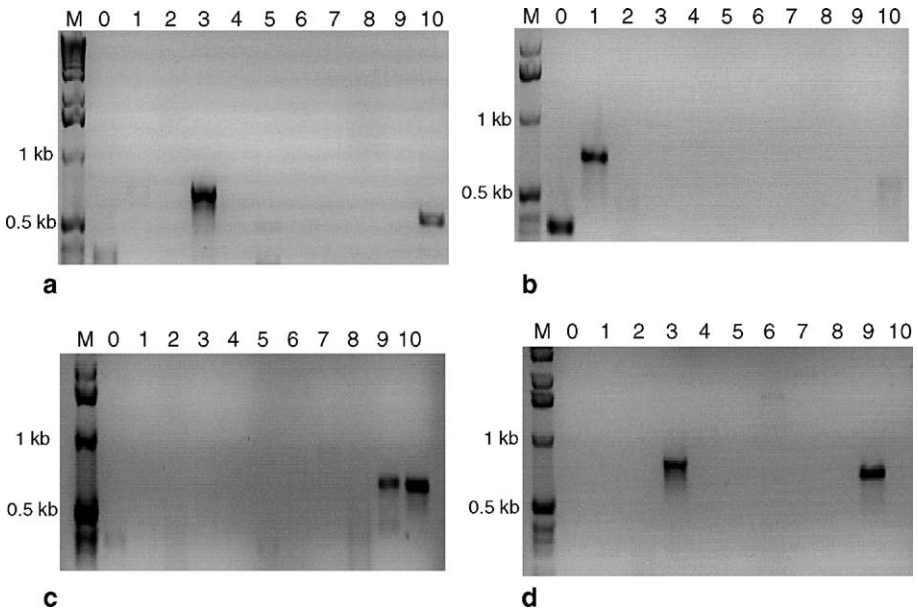

Fig. 3. RT-PCR analysis of aquaporin expression in primary cultures of cells from human epidermis. M: size marker. 0–10: DNA transcribed from cell culture mRNA was amplified by PCR with primers specific for human AQP0 to AQP10. (a) Human keratinocytes expressed both AQP3 and AQP10 mRNA (781 and 651 bp fragments respectively). (b) Melanocytes showed a signal for AQP1 (711 bp). (c) Monocytes showed a signal for AQP9 and AQP10 (664 and 651 bp respectively), but monocyte-derived dendritic cells used as a model of Langerhans cells showed AQP3 and AQP9 instead (d) (781 and 664 bp respectively).

Other than in melanocytes, aquaporins in the epidermis are therefore aquaglyceroporins (AQP3, 9, 10). In monocyte-derived dendritic cells (DC) used as models of Langerhans cells, aquaglyceroporins were reported to be involved in macropinocytosis [4]. Macropinocytosis is either constitutive or transiently induced in DCs and allows the capture of macrosolutes prior to the presentation of antigens. It was estimated that a DC can uptake the equivalent of its volume in an hour. de Baey and Lanzavecchia [4] reported that inhibition of DC AQPs by pCMBS, an inhibitor of most AQPs, inhibited fluid-phase endocytosis. They concluded that AQPs in dendritic cells are likely involved in transport of water coming from fluid-phase endocytosis out of the cell and thus in cell volume regulation.

Keratinocytes are by far the major cell type (~95% of cells) of the epidermis. As previously reported [24], we found AQP3 in human skin keratinocytes, but also AQP10, which is a new finding. AQP10 was identified recently [14], and it was not included in previous studies of AQPs expressed in mouse epidermis [18]. In addition, mouse AQP10 is a pseudo gene and the protein is not expressed [20]. Thus, little is known so far on AQP10 and the expression and localization of AQP10 in human epidermis will be interesting to study. However, human AQP10 has two isoforms [17] and the only antibodies currently available are against the unspliced form which lacks function and may not be the physiologically relevant human isoform (K. Ishibashi, personal communication). In any case, AQP10 is believed to share the functional properties of AQP3 (aquaglyceroporin), which is abundant in human keratinocytes. Therefore, the study of AQP3 function alone can largely account for the function of aquaglyceroporins in the epidermis.

3.3. Physiological role of AQP3 in skin epidermis

Fig. 4 summarizes features of AQP3 in human skin [see 24]: AQP3 is a ~26 kDa protein, mostly glycosylated (Fig. 4a). In *Xenopus* oocytes, a useful heterologous expression system for the study of AQP function, human AQP3 functions as a water and glycerol channel (Fig. 4b). In human skin, AQP3 is abundantly expressed in keratinocytes in the epidermis basal layers, and the stratum spinosum, but AQP3 disappears in the stratum granulosum and is totally absent in the stratum corneum (Fig. 4c). Fig. 4d shows a negative control of AQP3 staining in human epidermis. Finally, AQP3 localization to keratinocyte plasma membranes was confirmed at the EM level (Fig. 4e, stratum spinosum shown). We showed that AQP3 in human skin is a functional pH-sensitive water channel [24]. AQP-mediated transepidermal water loss was also suggested [2].

3.4. AQP3 as a glycerol transporter in mouse epidermis

Mice lacking AQP3 have dry skin [18]. Both water and glycerol contents are lower in the SC, and SC elasticity, barrier function and epidermis wound healing are impaired. But the analysis of skin phenotype in AQP3-null mice has progressively evolved to the conclusion that the glycerol, rather than the water transporting function of AQP3 is important in skin physiology [10,11,12]. The key findings in support of this conclusion in this model are that: i) skin dryness is not corrected by occlusion or exposure to humid atmosphere [18] and ii) glycerol replacement corrects the dry skin phenotype, i.e., low SC water content, decreased elasticity and reduced barrier function [11].

In short, glycerol replacement, but not water replacement corrects the skin phenotype of AQP3-null mice. Accordingly,

water immersion is known to impair the SC barrier [5,27], whereas glycerol has been known as an excellent chemical to improve skin hydration for centuries and has been widely used in cosmetics. The role of AQP3 in glycerol transport, has rapidly awakened interest for the metabolism of glycerol in skin. Using sebaceous gland deficient mice, Fluhr et al. reported that sebaceous-gland-derived glycerol is a major contributor to SC hydration [7]. Then, Choi et al. [5] confirmed the relationship between sebaceous-gland-derived glycerol and SC hydration. They demonstrated that SC glycerol extraction, by water immersion, correlates with a decrease in SC hydration. Further, Zheng et al. [28] suggested that AQP3 was colocalized with phospholipase D2 in lipid rafts of keratinocytes, where it would facilitate glycerol transport to phospholipase D2 for the synthesis of bioactive phosphatidylglycerol. Thus, AQP3 would regulate keratinocyte proliferation and differentiation.

In the literature, the relationship between SC hydration and glycerol is compelling:

- when AQP3 is present, either endogenous glycerol or exogenous glycerol improves SC hydration and SC water contents [5,7,11].
- When AQP3 is absent, SC glycerol, SC water contents are low and SC hydration is impaired [10,11,18].
- When AQP3 is absent, exogenous glycerol improves SC hydration and SC water contents [11].

Yet in these studies, the effectiveness of glycerol in SC hydration is independent of AQP3. Indeed, the “glycerol

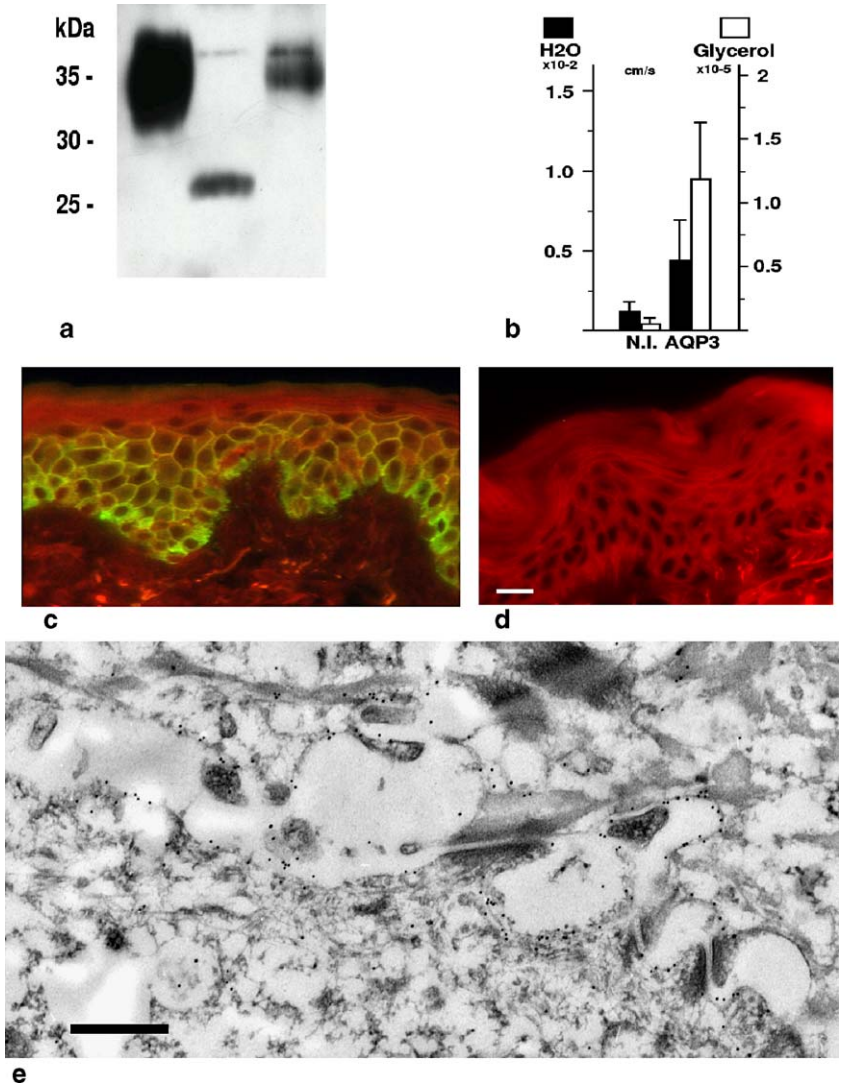

Fig. 4. Summary of AQP3 features in human skin. [see 24]] (a) Aquaporin-3 is a ~26 kDa protein (middle lane), which is mostly glycosylated in humans and shows an apparent mass of ~35 kDa (left and right lanes). The signal at 26 kDa was obtained by deglycosylation in PNGase F for 18 h. The right lane shows a sample also treated for 18 h, in the absence of enzyme. (b) Human AQP3 protein is expressed in *Xenopus* oocytes following the injection of 10 ng mRNA. AQP3 confers a high water and glycerol permeability to oocytes in comparison to non-injected oocytes (N.I.). (c) By indirect immunofluorescence in cryosections of human epidermis, AQP3 is localized to keratinocytes membranes from the basal layer up to the stratum spinosum. AQP3 is absent from the stratum granulosum and the stratum corneum. (d) Negative control for AQP3 staining in human epidermis. Scale bar=10 μm (e) by EM-gold labeling of AQP3, gold particules decorate the plasma membrane of keratinocytes between desmosomes (scale bar=0.5 μm).

replacement" strategy (exogenous glycerol administration) corrected SC hydration in AQP3-deficient mice, but it also improved SC hydration in wild-type mice [11]. In short, mice lacking AQP3 have dry skin and skin hydration can always be improved by glycerol, whether or not AQP3 is present. Therefore, we are still left with the question: which of water transport, glycerol transport, or both, is the physiological role of AQP3 in skin epidermis?

4. AQP3 function in human skin

Our laboratory has been most interested in aquaporins of human skin. There are at least two striking differences between human and mouse epidermis with respect to AQP3: i) the epidermis of mouse skin (Fig. 5a) is markedly thinner than human epidermis (Fig. 4c). ii) in mouse, AQP3 expression is largely restricted to the basal layer of the epidermis (Fig. 5a), whereas AQP3 is abundant in several layers of human epidermis up to the stratum granulosum (Fig. 4c). In addition to differences between species, AQP3 is never present in the SC (Figs. 4c, 5a). Thus, the SC is not the first site to look at for an AQP3-related phenotype. To address these issues, we decided to examine the consequences of AQP3 deletion at its actual site of expression in a thick epidermis, like the human epidermis. Repeated tape-stripping is an established procedure to induce epidermis hyperplasia [9]. As illustrated in Fig. 5, daily tape-stripping of the same region of skin over 8 days resulted in a significant increase in epidermis thickness, both in wild-type and AQP3-null mice (Fig. 5c, d). Following 8-daily strippings, AQP3 expression was no longer restricted to the basal layer of the epidermis (Fig. 5c, vs. a), but its distribution strikingly resembled that of AQP3 in human skin (Fig. 5c, vs. Fig. 4c). As expected, AQP3 was absent from skin in AQP3-deficient mice before and after stripping (Fig. 5b, d), but no other difference was apparent at the resolution of light microscopy. As previously reported [18], AQP3 deletion had no apparent effect on the epidermis structure at the electron-microscope level (Fig. 6b, vs. a). However, the hyperplastic epidermis of mice lacking AQP3 showed dilated intercellular spaces (Fig. 6d, *), which were not seen in the epidermis of wild-type mice (Fig. 6c). Such intercellular edema strongly suggests a defect in fluid movements, therefore in the movement of water, in the epidermis of AQP3-null mice. Thus, in addition to the mild dry skin phenotype previously observed [10–12,18], we report here for the first time that in a thick epidermis, like the human epidermis, AQP3 deletion can result in impaired water movements in viable layers of the epidermis, where AQP3 is normally expressed. This defect yields intercellular edema (spongiosis). This phenotype is likely more severe than the dry skin phenotype reported so far for AQP3 deletion.

We do not have access to patients lacking AQP3 to study their skin phenotype, but skin epidermis spongiosis is commonly associated to eczema. Therefore, we decided to examine whether intercellular edema in patients suffering from eczema was associated to a defect in plasma membrane AQP3. Fig. 7 shows the immunolocalization of AQP3 in biopsies from three different patients suffering from eczema. In patient (I) from Fig. 7a, the epidermis exhibited severe spongiosis and no AQP3 was detectable. In patient (II) from Fig. 7b, both healthy and damaged regions of the epidermis were seen on the same tissue section. In patient (III) (Fig. 7c) no alteration of the epidermis structure was detected. Accordingly, AQP3 expression and localization was unaltered. Thus, in all three

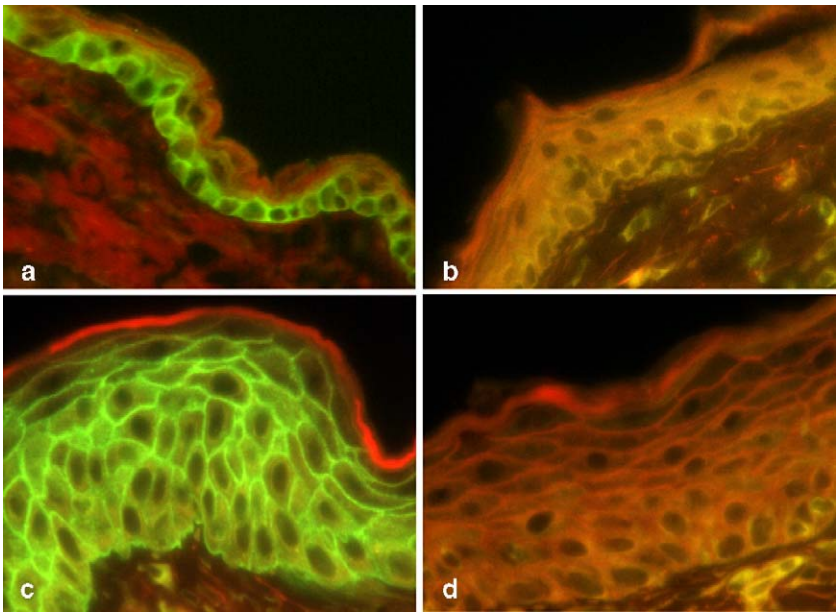

Fig. 5. AQP3 in mouse skin [see 18] a) by indirect immunofluorescence in cryosections, AQP3 is localized to keratinocytes membranes in the basal layer of the epidermis. (b) No staining for AQP3 was detected in transgenic mice deleted for AQP3. (c, d) Hyperplasia is obtained after 8 daily tape-stripping. (c) In wild type mice, AQP3 expression now extends on several layers of the epidermis, not just the basal layer. (d) Hyperplasia also occurs in mice lacking AQP3. No difference in skin structure is visible between genotypes at the light microscopy level.

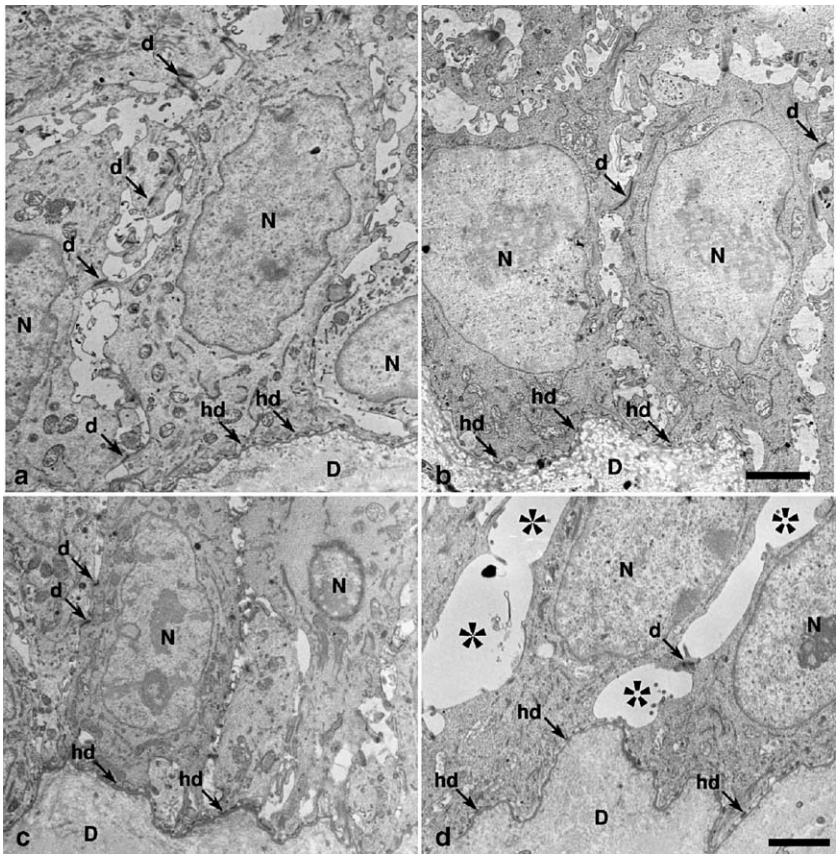

Fig. 6. Structure of the epidermis at the EM level. In normal (thin) epidermis, there is no difference in the epidermis of (a) wild-type and (b) AQP3-null mice. Following induction of hyperplasia by tape-stripping, the structure of wild-type mice (c) epidermis remains normal at the EM level, but transgenic mice lacking AQP3 (d) show marked intercellular edema (*). N: nuclei, d: desmosomes, D: dermis, scale bar = 1 μm.

patients, AQP3 was normally expressed when the epidermis was normal (Fig. 7b, c) but the water channel was totally absent from regions where intercellular edema was seen (Fig. 7a, b, arrows). Therefore, a possible relationship between the absence of AQP3 and intercellular edema, is suggested in these cases of eczema in human skin. It is very likely that AQP3 downregulation is only an indirect consequence of eczema and there is no evidence that the absence of AQP3 is responsible for the spongiosis. but the hypothesis that defective AQP3 is associated to defective water homeostasis and intercellular edema in the epidermis is consistent with observations in both mouse and human models. This hypothesis gives new directions for future research on water handling in the epidermis.

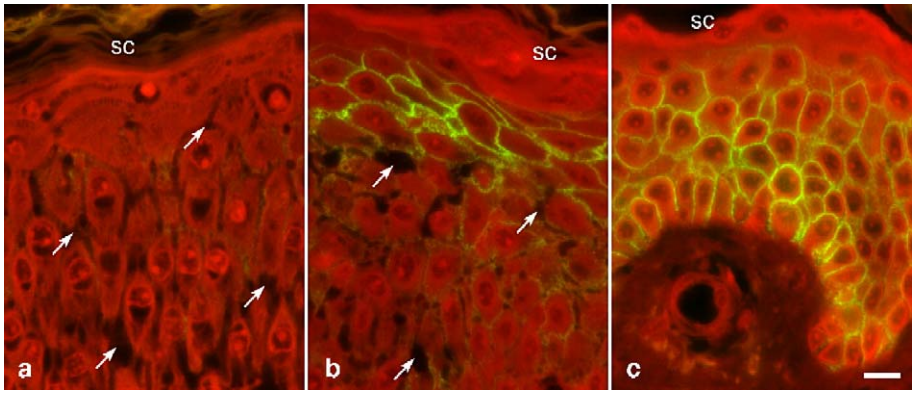

Fig. 7. AQP3 localization in skin biopsies from patients suffering from eczema and skin spongiosis (arrows). Antibody staining for AQP3 was not detected in the epidermis of one patient, with extensive spongiosis (arrows) (a). In the second patient (b), AQP3 staining was restricted to healthy regions of the epidermis. In the third patient, also suffering from eczema (c), no spongiosis was seen and AQP3 was evenly distributed in keratinocyte plasma membranes of the epidermis, like in normal epidermis. SC: stratum corneum, scale bar = 10 μm.

5. Conclusions

In conclusion, AQP1, 3, 5, 7, 9 and 10 may be expressed in human skin, but only AQPs of the epidermis, sweat glands and sebaceous glands are strictly related to skin physiology. In sweat glands AQP5 has been shown to be important for water secretion [21]. In the epidermis, we found AQP1 in melanocytes, but we confirm that AQP3 is the most abundant aquaporin [24], possibly along with AQP10. AQP3 is expressed in keratinocytes and has been attributed a role in the transport and metabolism of glycerol in mouse skin epidermis [10–12]. In the present study however, we would like to suggest that in human skin water transport by AQP3 is also important. Indeed, in two experimental models closely resembling human epidermis, we find that the absence of AQP3 is associated to intercellular edema (spongiosis). In addition, we demonstrate that a down regulation of AQP3 occurs in eczema.

References

[1] L. Abrami, F. Tacnet, P. Ripoche, Evidence for a glycerol pathway through aquaporin-1 (CHIP28) channels, Pflügers Arch Eur. J. Physiol. 430 (1995) 447–458.
[2] J. Agren, S. Zelenin, M. Hakansson, A.C. Eklof, A. Aperia, L.N. Nejsum, S. Nielsen, G. Sedin, Transepidermal water loss in developing rats: role of aquaporins in the immature skin, Pediatr. Res. 53 (2003) 558–565.
[3] D. Brown, A. Grosso, R.C. de Sousa, Isoproterenol-induced intramembrane particle aggregation and water flux in toad epidermis, Biochim. Biophys. Acta 596 (1980) 158–164.
[4] A. de Baey, A. Lanzavecchia, The role of aquaporins in dendritic cell macropinocytosis, J. Exp. Med. 191 (2000) 743–748.
[5] E.H. Choi, M.Q. Man, F. Wang, X. Zhang, B.E. Brown, K.R. Feingold, P.M. Elias, Is endogenous glycerol a determinant of stratum corneum hydration in humans? J. Invest. Dermatol. 125 (2005) 288–293.
[6] O. Devuyst, J. Ni, J.M. Verbavatz, Aquaporin-1 in the peritoneal membrane: implications for peritoneal dialysis and endothelial cell function, Biol. Cell 97 (2005) 667–673.
[7] J.W. Fluhr, M. Mao-Qiang, B.E. Brown, P.W. Wertz, D. Crumrine, J.P. Sundberg, K.R. Feingold, P.M. Elias, Glycerol regulates stratum corneum hydration in sebaceous gland deficient (asebia) mice, J. Invest. Dermatol. 120 (2003) 728–737.
[8] A. Frigeri, M.A. Gropper, F. Umenishi, M. Kawashima, D. Brown, A.S. Verkman, Localization of MIWC and GLIP water channel homologs in neuromuscular, epithelial and glandular tissues, J. Cell Sci. 108 (1995) 2993–3002.
[9] P.U. Giacomoni, Onc-gene expression in hyperplasia induced by tape stripping or by topical application of TPA, Br. J. Dermatol. 115 (Suppl. 31) (1986) 128–132.
[10] M. Hara, T. Ma, A.S. Verkman, Selectively reduced glycerol in skin of aquaporin-3-deficient mice may account for impaired skin hydration, elasticity, and barrier recovery, J. Biol. Chem. 277 (2002) 46616–46621.
[11] M. Hara, A.S. Verkman, Glycerol replacement corrects defective skin hydration, elasticity, and barrier function in aquaporin-3-deficient mice, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 7360–7365.
[12] M. Hara-Chikuma, A.S. Verkman, Aquaporin-3 functions as a glycerol transporter in mammalian skin, Biol. Cell 97 (2005) 479–486.
[13] M. Hara-Chikuma, E. Sohara, T. Rai, M. Ikawa, M. Okabe, S. Sasaki, S. Uchida, A.S. Verkman, Progressive adipocyte hypertrophy in aquaporin-7-deficient mice: adipocyte glycerol permeability as a novel regulator of fat accumulation, J. Biol. Chem. 280 (2005) 15493–15496.
[14] S. Hatakeyama, Y. Yoshida, T. Tani, Y. Koyama, K. Nihei, K. Ohshiro, J.I. Kamiie, E. Yaoita, T. Suda, K. Hatakeyama, T. Yamamoto, Cloning of a new aquaporin (AQP10) abundantly expressed in duodenum and jejunum, Biochem. Biophys. Res. Commun. 287 (2001) 814–819.
[15] T. Hibuse, N. Maeda, T. Funahashi, K. Yamamoto, A. Nagasawa, W. Mizunoya, K. Kishida, K. Inoue, H. Kuriyama, T. Nakamura, T. Fushiki, S. Kihara, I. Shimomura, Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 10993–10998.
[16] V. Leitch, P. Agre, L.S. King, Altered ubiquitination and stability of aquaporin-1 in hypertonic stress, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 2894–2898.
[17] H. Li, J. Kamiie, Y. Morishita, Y. Yoshida, E. Yaoita, K. Ishibashi, T. Yamamoto, Expression and localization of two isoforms of AQP10 in human small intestine, Biol. Cell 97 (2005) 823–829.
[18] T. Ma, M. Hara, R. Sougrat, J.M. Verbavatz, A.S. Verkman, Impaired stratum corneum hydration in mice lacking epidermal water channel aquaporin-3, J. Biol. Chem. 277 (2002) 17147–17153.
[19] A. Mobasheri, D. Marples, Expression of the AQP-1 water channel in normal human tissues: a semiquantitative study using tissue microarray technology, Am. J. Physiol.: Cell Physiol. 286 (2004) C529–C5237.
[20] T. Morinaga, M. Nakakoshi, A. Hirao, M. Imai, K. Ishibashi, Mouse aquaporin 10 gene (AQP10) is a pseudogene, Biochem. Biophys. Res. Commun. 294 (2002) 630–634.
[21] L.N. Nejsum, T.H. Kwon, U.B. Jensen, O. Fumagalli, J. Frokiaer, C.M. Krane, A.G. Menon, L.S. King, P.C. Agre, S. Nielsen, Functional requirement of aquaporin-5 in plasma membranes of sweat glands, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 511–516.
[22] S. Nielsen, B.L. Smith, E.I. Christensen, P. Agre, Distribution of the aquaporin CHIP in secretory and resorptive epithelia and capillary endothelia, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 7275–7279.
[23] Y. Song, N. Sonawane, A.S. Verkman, Localization of aquaporin-5 in sweat glands and functional analysis using knockout mice, J. Physiol. 541 (2002) 561–568.
[24] R. Sougrat, M. Morand, C. Gondran, P. Barre, R. Gobin, F. Bonte, M. Dumas, J.M. Verbavatz, Functional expression of AQP3 in human skin epidermis and reconstructed epidermis, J. Invest. Dermatol. 118 (2002) 678–685.
[25] Y. Sugiyama, Y. Ota, M. Hara, S. Inoue, Osmotic stress up-regulates aquaporin-3 gene expression in cultured human keratinocytes, Biochim. Biophys. Acta 1522 (2001) 82–88.
[26] R.R. Warner, M.C. Myers, D.A. Taylor, Electron probe analysis of human skin: determination of the water concentration profile, J. Invest. Dermatol. 90 (1988) 218–224.
[27] R.R. Warner, K.J. Stone, Y.L. Boissy, Hydration disrupts human stratum corneum ultrastructure, J. Invest. Dermatol. 120 (2003) 275–284.
[28] X. Zheng, W. Bollinger Bollag, Aquaporin 3 colocates with phospholipase d2 in caveolin-rich membrane microdomains and is downregulated upon keratinocyte differentiation, J. Invest. Dermatol. 121 (2003) 1487–1495.