American Journal of Clinical Patology (2019): 15 july of 2019

DOI: https://doi.org/10.1093/ajcp/aqz066

Expression pattern of Aquaporin-1 and Aquaporin-3 in melanocytic and non-melanocytic skin tumors

Giovana Osorio, MD, PhD, [1, 2] Teresa Zulueta-Dorado, MD, PhD, [3] Patricia González-Rodríguez, PhD, [1] José Bernabéu-Wittel, MD, PhD, [2] Julian Conejo- Mir, MD, PhD, [2] Reposo Ramírez-Lorca, PhD [1] and Miriam Echevarría, PhD, [1, *].

[1] Instituto de Biomedicina de Sevilla (IBiS) [Seville Biomedicine Institute], Hospital Universitario Virgen del Rocío [Virgen del Rocio University Hospital]/CSIC/Universidad de Sevilla [University of Seville], Department of Medical Physiology and Biophysics, Seville, Spain.

[2] Hospital Universitario Virgen del Rocío. Dermatology Department. Seville, Spain.

[3] Hospital Universitario Virgen del Rocío. Pathological Anatomy Department, Seville, Spain.

[*] **AUTHOR FOR CORRESPONDENCE:**

Miriam Echevarría Irusta

Instituto de Biomedicina de Sevilla (IBIS), Hospital Universitario Virgen del Rocío/ CSIC/ Universidad de Sevilla, Av. Manuel Siurot s/n, Sevilla 41013, Spain

Tel: +34 955 923036

Fax: +34 955 923101

E-mail address: irusta@us.es

ORCID: http://orcid.org/0000-0002-4758-9428

RUNNING TITLE: Aquaporins in skin tumors

KEY WORDS:

Aquaporin

Melanoma

Basal Cell Carcinoma

Squamous Cell Carcinoma

Immunohistochemistry

ABSTRACT

Objective: Study of AQP1 and AQP3 expression to understand its potential role in the pathophysiology of skin cancer.

Methods: Analysis of AQP1 and AQP3 expression by immunohistochemistry of 72 skin biopsies from melanocytic skin tumors, nonmelanocytic tumors or healthy samples.

Results: AQP1 showed strong labeling in 100% of benign common melanocytic nevi. Small blood vessels, stroma and melanophages surrounding different types of melanomas tumors also showed positive. Tumoral melanocytes in atypical nevi and melanomas were negative for AQP1.

AQP3 showed strong labeling in 100% of melanocytic nevi, 100% of atypical melanocytic nevi and 100% of melanomas. In all basal cell carcinoma and squamous cell carcinoma staining for AQP3 resulted positive.

Conclusion: This work represents the first demonstration of AQP1/AQP3 expression in human melanocytic skin tumors. More studies are needed to understand the underlying molecular mechanisms of expression of both AQPs in melanocytic tumors and their potential as molecular therapeutic targets.

Key points:

- AQP1 and AQP3 participate in the development of skin tumors.

- AQP1 is relevant for the angiogenesis necessary to sustain tumor cells and for the tumor's inherent inflammatory process.

- AQP3 overexpression in these types of skin tumors confers a greater proliferative capacity to the tumor cell.

1 INTRODUCTION

The skin is the largest organ in the body, and in addition to forming a natural barrier, it plays several essential roles in maintaining the body's homeostasis. Thus, the skin helps to keep the fluid and electrolyte balance, modulates body temperature, helps to maintain blood volume and possesses an important neuro receptor and immune system that governs the relationship with our surroundings. The three most common types of skin cancer are basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and melanoma. The incidence of skin tumors in general has increased markedly over the past 40 years, making it a public health problem.

AQP expression has been identified in various skin structures[1]. Specifically, AQP1 has been found in the endothelium of dermal capillaries and in cultured melanocytes and fibroblasts, although, curiously, its presence in these types of cells has not been confirmed *in vivo*. Similarly, AQP3 has been found in keratinocytes, fibroblasts, sebaceous glands and Langerhans cells; AQP5 in sweat glands; AQP7 in hypodermal adipocytes, AQP9 in keratinocytes and Langerhans cells and AQP10 in keratinocytes. However, the specific roles played by many of these proteins in the skin remains uncertain.

In terms of pathology, AQP-mediated water transport has been associated with the neoangiogenesis, migration, degree of invasion and metastatic potential of tumor cells, including the skin. Thus, AQP1 is linked to the production of tumoral neovessels, essential for the survival of cancer cells[2]. The results of animal studies have suggested a link between AQP1 and the

progression of melanoma[3,4]; however, it remains to be demonstrated whether AQP1 is typically expressed in human melanomas *in vivo*.

There is a significant amount of evidence linking changes in AQP3 expression with tumor growth[5], and particularly in skin cancer AQP3 seems to play a central role. Epidermal growth factor (EGF) was found to induce AQP3 expression via the MEK/ERK (*mitogen-activated protein kinase*/*extracellular signal-regulated kinase*) pathway, and to increase the migration and proliferation capacity of cells in two gastric carcinoma cell lines, which suggests that AQP3 may play an important role in tumor growth and dissemination[6]. Marked AQP3 expression has also been found in human squamous cell carcinoma[7] and in cell lines with epidermoid differentiation[8]. AQP3 is related to wound healing, lipid metabolism in the skin and regulation of the proliferation and differentiation of keratinocytes, with these latter properties being attributed to its ability to transport glycerol[9,10].

In light of the above, we hereby present an in-depth study of the expression of two AQPs, namely AQP1 and AQP3, which are known to be closely related to cancer, in an attempt to understand their possible role in the pathology of this disease and their potential use as specific therapeutic and diagnostic targets in skin cancer.

2 MATERIALS AND METHOD

2.1 Biopsies from skin tumors

In this study we performed immunohistochemical analysis of AQP1 and AQP3 expression on tissue sections of biopsies from 70 patients with various skin tumors, as shown in Table 1. Samples of all tumors were collected by surgery and kept as paraffin-embedded samples within the Department of Pathology, Hospital Universitario Virgen del Rocío (HUVR) [Virgen del Rocio University Hospital], Seville, Spain. Normal skin samples from healthy borders around the lesions were also analyzed. The tumors were staged according to the tumor-node-metastasis (TMN) classification system, and histologically classified according to the World Health Organization guidelines [11]. Tissue slides were hematoxylin-eosin stained and evaluated for diagnosis by two independent pathologists.

Written informed consent was obtained from all participants. The study followed the tenets of the WMA Declaration of Helsinki for research on human subjects and was approved by the ethics committee of HUVR.

2.2 Immunohistochemistry.

In order to set the immunohistochemistry conditions to detect AQP1 and AQP3, different dilutions of the antibody and developing times were tested on skin samples. All samples examined were obtained from formalin-fixed, paraffin-embedded pieces.

Tissue slices of 2-5 μm were cut with a microtome and mounted on microscope slides. The Immunohistochemical procedure started by removing the paraffin from the samples by immersion in xylene and rehydration through a series of decreasing dilutions of ethanol. Blocking of endogenous peroxidase activity was done by pre-incubation of samples in 3% H_2O_2. Heat-induced epitope retrieval was carried out by incubation of tissue sections at 65 ºC for 1 hour in sodium citrate (10 mM, pH: 6) and then blocking of unspecific binding was done by incubation for 1 hour in a PBS solution with 10% bovine serum and triton X-100 at 0.3%. Rabbit polyclonal anti-AQP1 (Abcam Cat # ab15080, RRID: AB_2056839, 1:500 dilution), or anti-AQP3 (Santa Cruz Biotechnology Cat # sc-20811, RRID: AB_2059551, 1:50 dilution) were used. The next two steps were EnVision + Dual Link System-HRP (DakoCytomation, Dako DenmarK) that contains goat anti-rabbit immunoglobulins conjugated to peroxidase-labeled polymer and DAB-substrate-DAB-chromogen for the development of brown precipitates. Qualitative analysis of signal level was performed according to the following criteria: absence of brown precipitate indicated a “negative” result for immunoreactivity and the presence of brown precipitate over specific cell areas was considered a “positive” result. Counter-staining with hematoxylin was done and sections were analyzed side by side with the pathologist by two independent observers. Sections were photographed using an AX70-Olympus microscope equipped with an Olympus DP10 camera. Omitting the primary antibody produced no staining.

2.3 Statistical analysis

Data analysis was performed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois), version 16.0. Continuous variables were described with the mean and standard deviation. Categorical data were summarized with the absolute and relative frequencies of each category. The expression of AQPs for each neoplasm and anatomical location was analyzed by χ^2 or Fisher exact test as appropriate. Alpha error was set at 0.05.

3 RESULTS

3.1 Population-based characterization of samples from primary skin tumors

The mean age of the patients from whom the 70 samples analyzed were obtained was 60 years (range: 21-89), excluding the controls. 38 (54.3%) of the patients were males and 32 (45.7%) were females. A total of 68 samples (97.1%) were primary tumors, with the remaining 2.9% (N = 2) corresponding to relapses. The most common location was the head and neck (45.7%, N = 32), followed by the trunk (30%, N = 21), upper extremities (15.7%, N = 11) and lower extremities (8.6%, N = 6). A more detailed description of the patients' demographic characteristics and the various biopsy subtypes is provided in Table 1 and Table 2 and in the supplementary material.

3.2 Expression of AQP1 and AQP3 in healthy skin

In healthy skin, as can be observed in Figure 1, AQP1 is expressed in the vascular endothelium and erythrocytes. The eccrine glands, which are secretory epithelia and therefore experience significant water flow, and dermal fibroblasts, were also positive for AQP1 (Table 2). In contrast, expression of AQP3 was restricted to the epidermal keratinocytes, with the intensity thereof being higher in the stratum basale and decreasing towards the more superficial layers of the skin. Keratinocytes in pilosebaceous follicles were also immunoreactive for AQP3, as were the eccrine and apocrine glands. Finally, fibroblasts, blood vessels and erythrocytes also stained positive for AQP3 (Figure 1). The

staining patterns for AQP1 and AQP3 in the different cells found in normal skin are summarized in Table 2.

3.3 Expression of AQP1 and AQP3 in tumors

92.9% (N = 65) of the 70 tumors evaluated were negative for AQP1, with a further 1.4% (N = 1) exhibiting focal immunoreactivity and 5.7% (N = 4) diffuse staining. All AQP1-positive samples corresponded to common melanocytic nevi. Lesion size was inversely related to signal intensity in all cases ($p = 0.01$). With respect to AQP3 immunostaining, 98.6% (N = 69) of tumors exhibited diffuse positive staining, with focal positive staining being observed in the remaining 1.4%. None of the tumors analyzed were negative in the immunohistochemical study for this protein. Lesion size was inversely related to signal intensity in all cases ($p = 0.01$).

3.3.1 AQP1 and AQP3 expression in common and atypical nevi

Immunostaining for AQP1 was intense in all common melanocytic nevi (100%, Figure 2), which is of interest given that the melanocytes in healthy skin were not positive and atypical or malignant melanocytic tumors were also negative for this protein (Table 2). Tumoral melanocytes were negative for AQP1 in all samples of atypical melanocytic nevi (100%, Figure 2). Positive staining for AQP1 was found in dermal capillaries and peritumoral connective tissue.

Although AQP3 expression has not been reported in either normal or proliferating melanocytes, staining of these cells was found in all common

melanocytic nevi analyzed in this study, as well as in all atypical nevi (Figure 2 and Table 2).

3.3.2 AQP1 and AQP3 expression in primary skin melanomas

As was the case for atypical melanocytic nevi, the tumoral melanocytes found in melanomas were all negative for AQP1. However, the small neovessels, peritumoral stroma and melanophages surrounding the tumor were immuno-positive for this protein (Figure 3).

It should be noted that positive staining for AQP1 was found in the neval cells from two melanoma samples, one was the superficial spreading type and the other was lentigo maligna, both of which were grew on a pre-existing neval lesion, although the melanoma cells themselves remained negative. This finding is consistent with the AQP1 staining pattern observed for each type of melanocytic tumor.

Similar to the situation for common and atypical nevi, AQP3 immunostaining was positive in all tumor cells from melanomas (Figure 3), independent of their histological sub-type (Table 2). These findings suggest that AQP3 may be a marker for proliferating melanocytes as this protein is found in all melanocytic tumoral lesions but is not expressed in basal melanocytes.

3.3.3 AQP1 and AQP3 expression in basal cell carcinomas and in squamous cell carcinomas

The tumor cells in all basal cell carcinomas studied were negative for AQP1, although marked AQP1 expression was observed in the stroma surrounding the tumor mass (Figure 4 and Table 2). In addition, AQP1

immunostaining was negative in all squamous cell carcinomas. However, positive staining for AQP1 was observed in the peritumoral stroma and in the neocapillaries and lymphocytes infiltrating this stroma.

High AQP3 expression was found in both basal cell carcinoma and squamous cell carcinoma cells, independently of the tumor sub-type (Figure 4). As both of these neoplasms are derived from epidermal keratinocytes, AQP3 expression in them was to be expected (Table 2).

4 DISCUSSION

The ongoing interest in the pathophysiological and molecular mechanisms underlying disease in general, and cancer in particular, has led to a rapidly growing field aimed at providing clinicians with the tools required to improve the diagnosis, treatment and/or prognosis of cancer. As a result, numerous specific therapeutic targets are now known. Aquaporins have provided new insight into the basic mechanisms underlying skin physiology and pathology and may therefore prove to be promising therapeutic targets in the diagnosis and treatment of skin cancer.

The analysis of healthy skin samples showed positive immunoreactivity for AQP1 in vascular endothelium and erythrocytes. Both eccrine and apocrine glands, which are secretory epithelia and therefore experience significant water flow, as well as dermal fibroblasts, were also positive. All these findings are consistent with previous studies in which AQP1 expression was shown in these tissues using the PCR technique and tissue microarrays[12] or immunohistochemistry[13]. However, in contrast to the findings published by other authors[1], we never detected AQP1 expression in melanocytes from the healthy skin samples analysed despite all our efforts to detect such positive immunoreactivity in this type of cell. The reason for this discrepancy remains unknown.

The expression pattern for AQP3 in healthy skin was very characteristic and in agreement with that reported previously[9, 14]. Thus, as expected, intense staining for AQP3 was observed in keratinocytes throughout the epidermis and around pilosebaceous follicles. Eccrine and apocrine glands, fibroblasts, blood

vessels and erythrocytes also stained positive for AQP3, whereas melanocytes did not exhibit expression of this protein in healthy skin.

The expression of both AQPs in tumor tissue was also very different and specific for each protein. Thus, none of the tumor types analyzed was found to express AQP1 in the carcinoma cells themselves, whereas all types of skin carcinoma studied exhibited marked AQP3 expression. This all-or-none expression pattern for these AQPs in the carcinomas studied herein limits their potential use as specific markers for this type of disease. However, this does not diminish interest in the potential roles played by each of these proteins in tumor development.

The tumor cells in all basal cell and squamous cell carcinomas and melanomas studied were negative for AQP1, although marked AQP1 expression was observed in the stroma surrounding the tumor masses, as well as in the neovessels and lymphocytes infiltrating this stroma. Another interesting observation was the intense staining of AQP1 observed in all common melanocytic nevi, but in none of the atypical melanocytic nevi. This unexpected AQP1 staining pattern is of particular interest given that melanocytes in healthy skin did not express AQP1 and neither did atypical or malignant melanocytic tumors. In addition, and to the best of our knowledge, there are no studies in the literature analyzing AQP1 expression in the human skin tumors studied herein, and there are no known precedents of an upregulation/downregulation pattern for AQP1 expression in any other type of tumors. In light of our findings, it would be tempting to propose that AQP1 plays a key role in the transformation of normal melanocytes into a benign neoplastic state (nevus), and that the subsequent malignant transformation is independent

of this protein. As such, it would be of interest to study the molecular basis underlying the regulation of this particular on/off process of AQP1 expression observed in melanocytic skin tumors.

The marked AQP1 expression observed in the peritumoral stroma, in fibroblasts and, in particular, in the vascular endothelium of neovessels surrounding and infiltrating the tumor mass is in agreement with previous findings in various other tumor types. Thus, AQP1 expression has been associated with carcinoma of the colon, breast, brain and lung, as well as with hemangioblastoma and multiple myeloma[15-21]. Animal studies have suggested a key role played by AQP1 in angiogenesis and cell migration within the vascular endothelium[2], which is fundamental for the formation of new vessels to supply the tumor. It has been reported that AQP1 localizes in the main border of membrane protrusions during cell migration and is associated with various transporters, Na/H^+ and Cl/HCO_3 exchangers[3], in keeping with the rapid movement of ions and water required during migration.

We also found the fibroblasts and lymphocytes surrounding tumor cells to be immunopositive for AQP1, which is consistent with a possible role for this protein in the inflammatory phenomena triggered by the immune system as part of its immunovigilance against tumors. The inflammatory infiltration and fibrosis surrounding the tumor also demonstrates the presence of this protein in those processes related to tumor development.

On the other hand, high expression of AQP3 was detected in tumor cells from both basal cell and squamous cell carcinoma in all samples evaluated in this study. Similarly, although AQP3 expression was not detected in melanocytes from healthy skin, intense staining of this protein was observed in

both benign and malignant melanocytic lesions. Some authors have suggested that AQP3 overexpression in tumors is related to the basal expression of this protein and the function of the tissue or organ[5]. However, there is little evidence for the validity of this hypothesis in melanomas as, under normal conditions, melanocytes do not express AQP3 and melanomas are not glandular-type tumors.

Previous studies have linked AQP3 to basal cell carcinoma and squamous cell carcinoma or epidermal tumors[22], where intense immunohistochemical staining was found in tumor areas of human primary squamous cell carcinoma such as esophageal and lingual cancers[22], and a higher incidence of AQP3 expression and H-score was found in SCC compared to BCC in human skin biopsies that were immunohistochemicaly analyzed[23]. Also, in cell lines with epidermoid differentiation (keratocarcinoma), endogenous AQP3 expression has been demonstrated at both the mRNA and protein levels[8], and evidence for the importance of AQP3 in tumor formation has also been obtained from animal models. The hypothesis that glycerol transport via AQP3 is a determining factor for proliferation and cutaneous carcinogenesis based on its role in cellular energy (ATP) production was postulated some time ago[10]. The incorporation of glycerol into lipid synthesis, ATP-facilitated MAP-kinase signaling and a positive–feedback loop through which cell proliferation increases AQP3 expression have also been proposed as part of the pathophysiological mechanism of AQP3[10]. Nowadays it is believed that AQP3 is involved in the migration, proliferation and invasion of skin tumors, as well as in the worsening of the epithelial-to-mesenchymal transition[24].

In conclusion, the results of this study are consistent with the involvement of both AQP1 and AQP3 in the development of skin tumors. AQP1 appears to be relevant for the neoangiogenesis required to sustain tumor cells and for the inflammatory process inherent to the tumor, which is in agreement with previous studies showing an association between AQP1 expression and the formation of new blood vessels as well as with a more aggressive and potentially metastatic profile in various types of human cancers[2,17,25-28]. Similarly, AQP3 overexpression in the three types of tumor analyzed, namely basal cell carcinoma, squamous cell carcinoma and melanoma, probably confers a greater proliferative capacity on the tumor cell[28,29], together with an increased passage of glycerol and/or solutes such as hydrogen peroxide (H_2O_2)[29], which increase the energy reservoir in the cell and/or the potentially carcinogenic oxidative stress inside the cell.

FUNDING

This study was supported by the Spanish Ministry of Economy and Competition, co-funded by the Instituto de Salud Carlos III [Charles III Institute of Health] and by FEDER *(*Fondo Europeo de Desarrollo Regional [European Fund for Regional Development], (grants number PI12/01882 and PI16/00493); the Regional Department of Health- Fundación Pública Andaluz Progreso y Salud [Andalusian Public Foundation for Progress and Health] (grant number PI 0018-2014) to RRL.

ACKNOWLEDGEMENTS

The authors would like to thank the Pathological AnatomyDepartment at Hospital Universitario Virgen del Rocío, for providing the biopsies collected and appropriately classified from patient donors.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest

REFERENCES

1. Boury-Jamot M, Sougrat R, Tailhardat M, et al. Expression and function of aquaporins in human skin: is aquaporin-3 just a glycerol transporter? Biochim Biophys Acta. 2006; 1758:1034-42.

2. Saadoun S, Papadopoulos M, Hara-Chikuma M, et al. Impairment of angiogenesis and cell migration by targeted Aquaporin-1 gene disruption. Nature. 2005; 434: 786-92.

3. Papadopoulos M, Verkman A. Aquaporins and cell migration. Pflugers Arch. 2008; 456:693-700.

4. Hu J, Verkman A. Increased migration and metastatic potential of tumor cells expressing aquaporin water channels. FASEB J. 2006; 20: 1892-4.

5. Liu YL, Matsuzaki T, Nakazawa T et al. Expression of aquaporin 3 (AQP3) in normal and neoplastic lung tissues. Hum Pathol. 2007; 38: 171-8.

6. Huang Y, Zhu Z, Sun M, et al. Critical role of aquaporin-3 in the human epidermal growth factor-induced migration and proliferation in the human gastric adenocarcinoma cells. Cancer Biol Ther. 2010; 9:1000-7.

7. Hara-Chikuma M, Verkman A. Roles of Aquaporin-3 in the epidermis. J Invest Dermatol. 2008; 128:2145-51.

8. Nakakoshi M, Morishita Y, Usui K, et al. Identification of a keratinocarcinoma cell line expressing AQP3. Biol Cell. 2006; 98:95-100.

9 Boury-Jamot M, Daraspe J, Bonte F, et al. Skin Aquaporins: function in hydration, wound healing, and skin epidermis homeostasis. Handb Exp Pharmacol. 2009; 190: 205-17.

10. Verkman A, Hara-Chikuma M, Papadopoulos M. Aquaporins - new players in cancer biology. J Mol Med. 2008; 86:523-9.

11. Travis W., Brambilla E., Müller-Hermelink H., Harris C., editors. (Eds). Pathology and Genetics of Tumors of the Lung, Pleura, Thymus and Heart. Lyon: IARC Press; 2004.

12. Mobasheri A, Marples D. Expression of the AQP1 water channel in normal tissues: a semiquantitative study using tissue microarray technology. Am J Physiol Cell Physiol. 2004 ; 286: C529-37.

13. Marchini G, Stabi B, Kanses K, et al. AQP1 and AQP3, psoriasin, and nitric oxide synthases 1-3 are inflammatory mediators in erythema toxicum neonatorum. Ped Dermatol. 2003; 20: 377-84.

14. Sougrat R, Morand M, Gondran C, et al. Functional expression of AQP3 in human skin epidermis and reconstructed epidermis. J Invest Dermatol. 2002; 118:678-85.

15. Moon C, Soria J, Jang S, *et al*. Involvement of aquaporins in colorectal carcinogenesis. Oncogene. 2003; 22: 6699–8703.

16. Endo M, Jain R, Witwer B, et al. Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas . Microvasc Res. 1999; 58: 89–98.

17. Saadoun S, Papadopoulos M, Davies D, et al. Increased Aquaporin-1 water cannel expression in human brain tumors. Br J Cancer. 2002; 87: 621-23.

18. Hoque M, Soria J, Woo J, *et al*. Aquaporin 1 is overexpressed in lung cancer and stimulates NIH-3T3 cell proliferation and anchorage-independent growth. Am. J. Pathol. 2001; 168: 1345–1353.

19 Chen Y, Tachibana O, Oda M, *et al*. Increased expression of aquaporin 1 in human hemangioblastomas and its correlation with cyst formation. J Neurooncol. 2006; 80: 219–225.

20. Vacca A, Frigeri A, Ribatti D, et al. Microvessel overexpression of aquaporin-1 parallels bone marrow angiogenesis in patients with active multiple myeloma. Br J Haematol. 2001; 113: 415-421.

21. López-Campos JL, Sánchez Silva R, Gómez Izquierdo L, et al. Overexpression of Aquaporin-1 in lung adenocarcinomas and pleural mesotheliomas. Histol Histopathol. 2011; 26: 451-9.

22. Kusayama M, Wada K, Nagata M, *et al*. Critical role of aquaporin 3 on growth of human esophageal and oral squamous cell carcinoma. Cancer Sci. 2011; 102(6):1128-36.

23. Seleit I, Bakry OA, Al Sharaky D, et al. Evaluation of Aquaporin-3 Role in Nonmelanoma Skin Cancer: An Immunohistochemical Study Ultrastruct. Pathol. 2015; 39(5):306-

24. Marlar S, Jensen H H, Login F H, et al. Aquaporin-3 in Cancer. Int. J. Mol. Sci. 2017, 18.

25. Oshio K, Binder D, Liang Y, et al. Expression of Aquaporin-1 water channel in human glial tumors. Neurosurgery. 2005; 56: 375-81.

26. Longatti P, Basaldella L, Orvieto E, et al. Aquaporin-1 expression in cystic hemangioblastomas. Neuroscience letters. 2006; 392: 178-180.

27. Galán-Cobo, A., Ramírez-Lorca, R., Toledo-Aral, J. J., and Echevarría, M. (2016). Aquaporin-1 plays important role in proliferation by affecting cell cycle progression. J. Cell. Physiol. 231, 243–256. doi: 10.1002/jcp.25078

28. Galan-Cobo A; Ramirez-Lorca R, Echevarria E. Role of aquaporins in cell proliferation: What else beyond water permeability? Channels. 2016; 10(3): 185–201

29. Galán-Cobo A, Ramírez-Lorca R, Serna A, et al. Overexpression of AQP3 Modifies the Cell Cycle and the Proliferation Rate of Mammalian Cells in Culture. PLoS One. 2015; 10(9):e0137692.

Table 1. Population-based characterization of the tissue samples included in the study.

Diagnosis	Age (years)	Size (cm)
Common melanocytic nevi		
• N	5	5
• Mean	31.40	0.460
• Standard Dev.	10.968	0.0548
• Median	29.00	0.500
• Minimum	21	0.4
• Maximum	50	0.5
Atypical nevi		
• N	15	15
• Mean	32.13	0.553
• Standard Dev.	6.334	0.1807
• Median	32.00	0.500
• Minimum	23	3
• Maximum	46	1.0
Melanoma		
• N	20	20
○ Lentigo Maligna	5	5
○ Superficial spreading	6	6
○ Nodular	5	5
○ Acral	4	4
• Mean	61.85	1.865
• Standard Dev.	6.334	1.2236
• Median	32	1.400
• Minimum	23	5
• Maximum	46	4.0
Basal cell carcinoma		
• N	15	15
• Mean	69.80	0.893
• Standard Dev.	13.127	0.4682
• Median	73.00	0.800
• Minimum	34	0.3
• Maximum	86	1.9
Squamous cell carcinoma		
• N	15	15
• Mean	76.40	1.507
• Standard Dev.	11.160	0.9520
• Median	84	1.200
• Minimum	54	0.5
• Maximum	89	3.5
TOTAL		
• N	70	70
• Mean	58.13	1.199
• Standard Dev.	21.682	0.9732
• Median	60.00	0.800
• Minimum	21	0.3
• Maximum	89	4.0

Table 2. Immunopositivity of AQP1 and AQP3 in different cell types of normal skin and in skin tumors.

Cell type	AQP1	AQP3
Keratinocytes	Negative	Positive
Melanocytes	Negative	Negative
Blood vessels	Positive	Positive
Fibroblasts	Positive	Positive
Eccrine glands	Positive	Positive
Apocrine glands	Positive	Positive
Hystologic type	**AQP1 (N)**	**AQP3 (N)**
Melanocytic nevus	Positive (5/5)	Positive (5/5)
Atypical melanocytic nevus	Negative (15/15)	Positive (15/15)
Melanoma	Negative (20/20)	Positive (20/20)
Basal cell carcinoma (BCC)	Negative (15/15)	Positive (15/15)
Squamous cell carcinoma (SCC	Negative (15/15)	Positive (15/15)

FIGURE CAPTIONS

Figure 1. Expression of AQP1 and AQP3 in healthy skin. (**A**) Negative staining for AQP1 in the epidermis, but positive staining in vascular endothelium (arrows) and fibroblasts (arrow heads). (**B**) Higher magnification of a small area shown in Figure **A**. (**C-D**) Positive staining for AQP3 in epidermal keratinocytes (arrows). Keratinocytes found in the pilosebaceous follicle were also immunoreactive for AQP3 (arrow head). (**E**) Higher magnification of epidermal keratinocytes showing AQP3 staining. Staining for AQP3 was observed in keratinocytes of apocrine (**F**) and eccrine (**G**) glands. Scale represented in Figure **A**: 50 μm, in Figures **B**, **E**, **F** and **G**: 100 μm, in Figure **D**: 200 μm and in Figure **C**: 250 μm.

Figure 2. AQP1 and AQP3 expression in common and atypical melanocytic nevi. (**A**) Epidermis is consistently negative but nevi cells are strongly positive for AQP1 (arrows). (**B**) Higher magnification of a small area shown in Figure **A**. (**C**) Positive staining for AQP3 is observed in entire epidermis and in all melanocytes of nevi theca cells. (**D**) Cells conforming nevi thecas are shown at larger magnification (arrows). (**E**) All melanocytic thecas are negative for AQP1. (**F**) Intense positive signal for AQP3 is observed in melanocytic nevus cells. Samples in **A-D** correspond to common melanocytic nevi biopsies, and **E** and **F** correspond to atypical melanocytic nevi. Scales in **A**, **C**, **E** and **F** represent 50 μm, and 200 μm in **B** and **D**.

Figure 3. AQP1 and AQP3 Expression in different types of melanomas: (**A** and **E**) Nodular melanoma, (**B** and **F**) Lentigo maligna melanoma, (**C** and **G**) Superficial spreading melanoma and (**D** and **H**) acral lentiginous melanoma. (**A-D**) All melanocytic cells are negative for AQP1 staining; however, positive staining was detected in neovessels inside the tumor area serving as an internal positive control. Staining in blood vessels is seen in (**A**), (**C**) and (**D**) (arrows) and melanophages (**B**) (arrow heads). (**E- H**) All melanocytic cells by contrast showed intense staining for AQP3. Photographs **A-D** are all at the same magnification. The scale shown in **D** represents 100 μm. In E, the scale represents 250 μm, and in **F**, **G** and **H it represents** 100 μm.

Figure 4. AQP1 and AQP3 Expression in basal cell carcinoma and squamous cell carcinoma. AQP1 expression was not detected in either the epidermis (**A**), or in tumor cells (**B**) but a strong positive signal was detected in the surrounding stromal tissue. A high rate of AQP3 expression was observed in basal cell carcinoma (**C**). Higher magnification of the tumor zone of **C** is shown (**D**). In squamous cell carcinoma, tumor cells were negative for AQP1 immunostaining, but capillaries in the surrounding stroma tissue (arrows) did express AQP1 in (**E**) and (**F**). Intense staining for AQP3 was seen in tumor cells of squamous cell carcinomas (**G**) and (**H**). The scale in **A** represents 400 μm, in **C**, **E** and **G** represents 200 μm and in **B**, **D**, **F** and **H** it represents 100 μm.

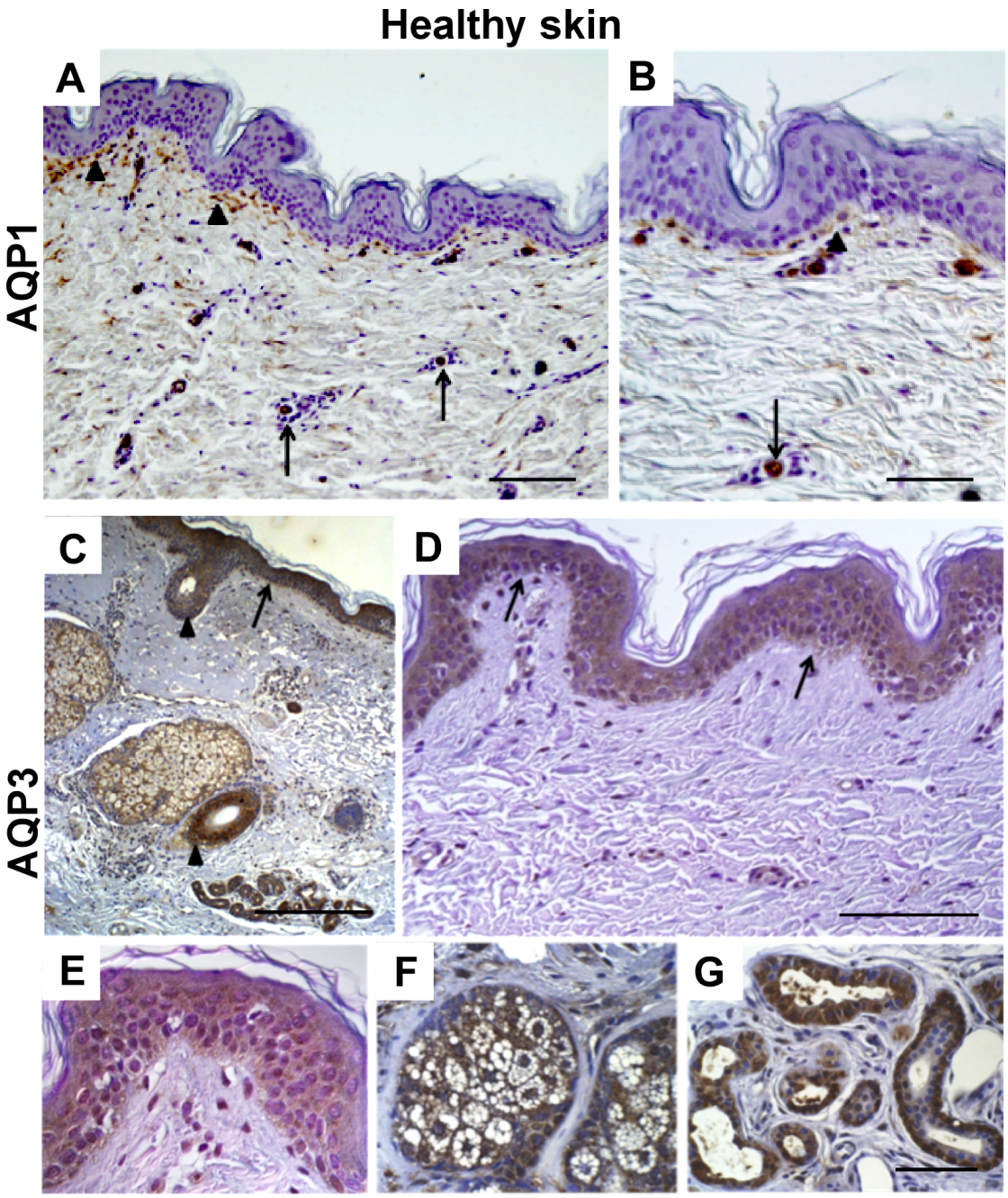

Figure 1.

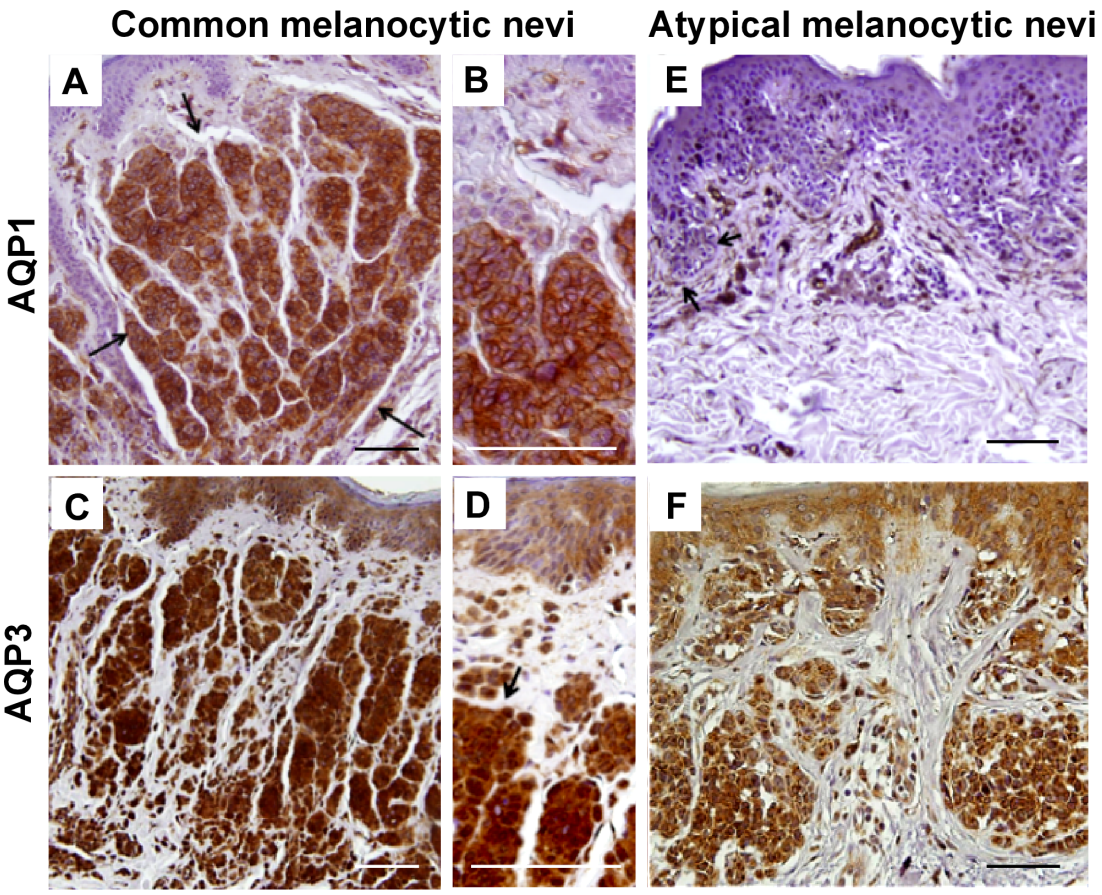

Figure 2.

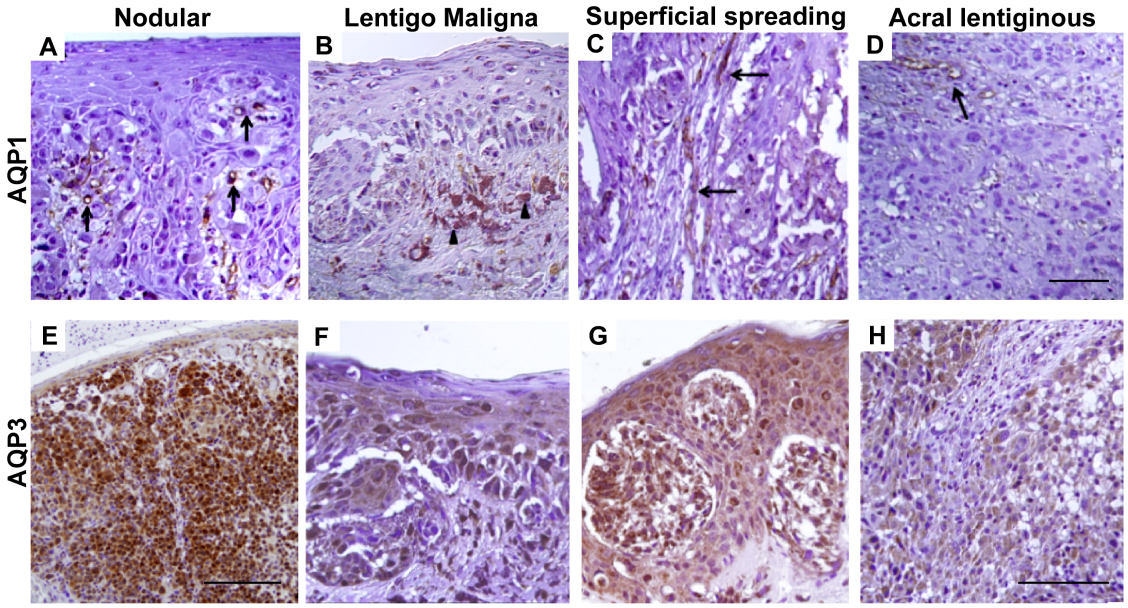

Figure 3.

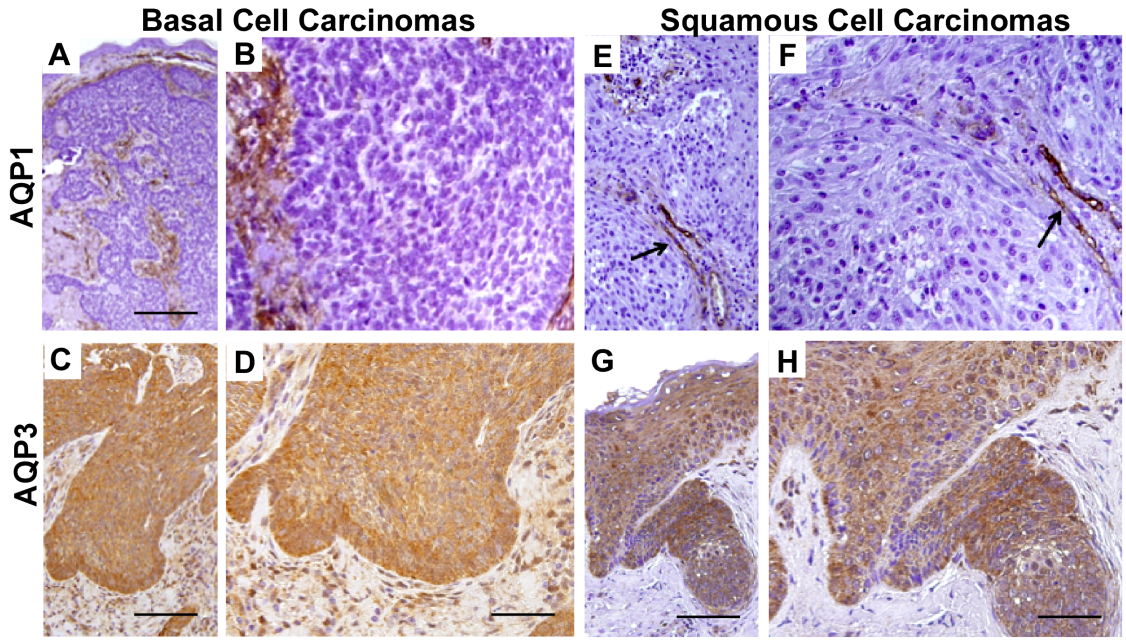
Basal Cell Carcinomas
Squamous Cell Carcinomas
A
B
E
F
AQP1
C
D
G
H
AQP3

Figure 4.