Expression and Significance of AQP3 in Cutaneous Lesions

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1. Introduction

Aquaporin 3 (AQP3) is a water-transporting aquaglyceroporin and plays a significant role in physiologic functions of reabsorption and secretion in the kidney [1, 2], epidermis [3, 4], pancreas [5], prostate [6], etc. The AQP3 protein expression was observed in some normal tissue such as pituitary cells, salivary gland, thymic epithelium, bronchial epithelial cells and pulmonary alveolar epithelium, pancreatic islet, and squamous epithelium of the esophagus, uterine cervix, skin [7], etc. Furthermore, AQP3 has been detected in malignancy of several organs including the colon [8], ovary [9], breast [10], pancreas [11], and prostate [12]. Expression of AQP3 in these tumors was reported to correlate with tumorigenesis, invasion, metastasis, and proliferation [8–13]. In a previous study, we found that AQP3 protein was widely distributed in neoplastic tissues including skin squamous cell carcinomas by immunohistochemical staining [7].

Skin cancer is one of the most common human malignancies with increasing incidence worldwide [14–16]. Skin cancer is mainly divided into two large groups: cutaneous melanoma and nonmelanoma skin tumor. Malignant melanoma is one of the most aggressive human cancers and causes 75% mortality in all skin cancer in the United States [17]. Nonmelanoma skin tumor accounts for nearly 95% of cutaneous neoplasms, and the most common lesions include basal cell carcinoma, squamous carcinoma, and sebaceous carcinoma [15]. Frequent exposure to ultraviolet radiation or sunlight acts as the major factor for both groups of skin cancer [14, 17]. With the development of clinical examination, such as dermoscopy and in vivo reflectance confocal microscopy, the diagnosis and differential diagnosis of skin cancer have become more precise.
In a recent study [7], we found that AQP3 protein was immunohistochemically diffusely positive in the cytoplasmic membrane of normal cutaneous cells including squamous epithelium, sudoriferous gland, sebaceous gland, and apocrine gland but not in melanocytes. We also found a high positive expression of AQP3 in skin squamous carcinomas. However, limited information on AQP3 expression in other skin tumors is available. In this study, we investigated the expression and significance of AQP3 in a large series of skin lesions, using RT-PCR and immunohistochemistry.

2. Materials and Methods

2.1. Case Selection. Our study was approved by the local research ethics committee of Peking University Cancer Hospital & Institute. We included 311 surgically resected skin lesions from the routine surgical pathology file of Peking University Cancer Hospital & Institute, including 74 benign nonneoplastic lesions (14 seborrheic keratoses, 16 verruca vulgaris, 13 sebaceous hyperplasias, 5 molluscum contagiosum, and 26 nevocellular nevi), 40 benign skin tumors (7 hидradenomas, 7 eccrine poromas, 16 sebaceousomas, and 10 sebaceous adenomas), and 197 premalignant lesion and malignant tumors (24 solar keratoses, 26 Bowen’s diseases, 43 squamous cell carcinomas, 32 basal cell carcinomas, 16 extramammary Paget’s disease, 9 sebaceous carcinomas, 19 apocrine carcinomas, and 28 malignant melanomas). The cases were summarized in Table 1. The diagnosis of all these cases was confirmed by 2 pathologists (DN and YB). The blocks for the study also included normal tissues adjacent to the lesions.

2.2. RNA Extraction and RT-PCR. Total RNA was isolated from various formalin-fixed and paraffin-embedded skin lesion tissues with Recover ALL™ Total Nucleic Acid Isolation Kit (Applied Biosystems, USA). We designed the specific PCR primers targeted for AQP3 and PGK1 (as an internal control) as shown in Table 2. HotstarTaq DNA polymerase kit (Qiagen, USA) was performed for amplification. PCR conditions were described previously [11].

2.3. Immunohistochemical Staining. The immunohistochemical staining was performed as described previously [7]. The rabbit polyclonal anti-AQP3 antibody was obtained from Sigma (HPA014924, Sigma, USA, 1:4000 dilution). The expression of AQP3 was evaluated as negative (<1% cells positive for AQP3), focal (1 to 9% of positive cells), intermediate (10 to 50% of positive cells), and diffuse (51% or more positive cells). Staining was individually evaluated by two observers (DN and YB) blinded to all clinicopathological information.

3. Results

3.1. AQP3 mRNA Expression in the Skin Lesions. We first detected and analyzed the expression pattern of the AQP3 mRNA in the normal squamous epithelium and skin lesions by RT-PCR. As shown in Figure 1, the bands of AQP3 were seen in normal skin tissues (NST), solar keratosis (SoK), seborrheic keratosis (SBK), eccrine poroma (EP), sebaceous adenomas (SB), squamous cell carcinoma (SCC), Bowen’s disease (BD), and apocrine carcinoma (AC) but not expressed in nevocellular nevus (NN), basal cell carcinoma (BC), and malignant melanoma (MM). These results were in accordance with our immunohistochemical observation.

3.2. AQP3 Expression in Normal Control Tissue. We used the sections from normal kidney tissue as a positive control. The expression of AQP3 was specifically localized in the cytoplasmic membrane of the collecting duct cells of the kidney (Figure 2(a)) and squamous cells of the skin (Figure 2(b)) as previously described [7].

3.3. AQP3 Expression in Benign Skin Lesions and Tumors. The immunohistochemical staining of AQP3 in skin lesions is listed in Table 1. Among the nonneoplastic skin lesions, all cases of seborrhic keratosis (n = 14), verruca vulgaris (n = 16), molluscum contagiosum (n = 5), and sebaceous hyperplasia (n = 13) showed diffuse staining for AQP3 (Figures 3(a)–3(d)). Among the 26 nevocellular nevi, 8 showed focal AQP3 staining and the remaining 18 were negative. Among the benign skin tumors, positive AQP3 staining was seen in all 7 hidradenomas (all diffuse), 6/7 eccrine poromas (all diffuse), all 16 sebaceousomas (4/16 focal, 4/16 intermediate, and 8/16 diffuse), and all 10 sebaceous adenomas (all diffuse).

3.4. AQP3 Expression in the Premalignant Lesion and Malignant Skin Tumors. The immunohistochemical results of AQP3 staining in premalignant solar keratoses and malignant skin tumors are summarized in Table 1. All 24 solar keratoses (Figure 4(a)) and 16 extramammary Paget’s diseases (Figure 4(c)) showed diffuse AQP3 staining. Positive AQP3 staining was seen in 26 Bowen’s diseases (Figure 4(b)) (2 focal, 24 diffuse), 43 squamous cell carcinomas (Figure 4(d)) (3 focal, 7 intermediate, and 33 diffuse), and 19 apocrine carcinomas (Figure 4(e)) (3 intermediate, 16 diffuse). All 32 basal cell carcinomas (Figure 4(f)), 9 sebaceous carcinomas (Figure 4(g)), and 28 malignant melanomas (Figure 4(h)) were negative for AQP3 staining.

4. Discussion

Aquaporin3 (AQP3), one of the aquaglyceroporins, is responsible for transporting water and glycerol and maintaining fluid homeostasis in normal tissues [18–20]. In our study, we found that most skin tumors, except basal cell carcinoma, sebaceous carcinoma, and melanoma, showed positive AQP3 staining (diffuse in most positive tumors). No AQP3 staining was seen in basal cell carcinomas, sebaceous carcinomas, and malignant melanomas. In our previous study [7], we showed that AQP3 staining was present in normal epidermal cells and sweat gland cells. Our findings support the view that the water homeostasis regulated by AQP3 was well maintained during carcinogenesis in most of the skin tumors except basal cell carcinoma, sebaceous carcinoma, and malignant melanoma.

No expression of AQP3 in basal cell carcinoma and sebaceous carcinoma might help to elucidate their histogenesis and/or pathogenesis. Basal cell carcinoma usually arises
from the lowermost layer of the epidermis and less commonly from the outer root sheath of the pilosebaceous unit. Basal cell carcinoma cells share many common features with follicular epithelium such as hair bulbs, follicular bulges, and follicular matrix cells [21]. In our previous study, we did not observe AQP3 expression in hair follicles [7]. No expression of AQP3 in basal cell carcinoma further confirms the hair follicle origin of basal cell carcinoma. Sebaceous carcinoma shows no expression of AQP3 whereas benign sebaceous lesions including sebaceous hyperplasia, sebaceous adenoma, and sebaceous hyperplasia all show retained expression of AQP3, suggesting that loss of AQP3 may contribute to the carcinogenesis of sebaceous carcinoma.

The absent expression of AQP3 in basal cell carcinoma and sebaceous carcinoma also has some diagnostic value. In clinical practice, sometimes, it is a big challenge to distinguish basal cell carcinoma from basaloid squamous cell carcinoma or metaplastic basal cell carcinoma from poorly differentiated squamous cell carcinoma. AQP3 immunohistochemical staining is helpful in this

Table 1: Summary of immunohistochemistry for AQP3 in skin lesions.

| Lesion Type                          | N  | Absent | Focal | Intermediate | Diffuse |
|-------------------------------------|----|--------|-------|--------------|---------|
| Nonneoplastic lesions               |    |        |       |              |         |
| Seborrheic keratosis                 | 14 | 0      | 0     | 0            | 14      |
| Verruca vulgaris                    | 16 | 0      | 0     | 0            | 16      |
| Sebaceous hyperplasia               | 13 | 0      | 0     | 0            | 13      |
| Molluscum contagiosum               | 5  | 0      | 0     | 0            | 5       |
| Nevocellular nevus                  | 26 | 18     | 8     | 0            | 0       |
| Benign tumors                       |    |        |       |              |         |
| Hidradenoma                         | 7  | 0      | 0     | 0            | 7       |
| Eccrine poroma                      | 7  | 1      | 0     | 0            | 6       |
| Sebaceousoma                        | 16 | 0      | 4     | 4            | 8       |
| Sebaceous adenoma                   | 10 | 0      | 0     | 0            | 10      |
| Premalignant lesion and malignant tumors |    |        |       |              |         |
| Solar keratosis                     | 24 | 0      | 0     | 0            | 24      |
| Bowen’s disease                     | 26 | 0      | 2     | 0            | 24      |
| Squamous cell carcinoma             | 43 | 0      | 3     | 7            | 33      |
| Basal cell carcinoma                | 32 | 32     | 0     | 0            | 0       |
| Paget’s disease                     | 16 | 0      | 0     | 0            | 16      |
| Sebaceous carcinoma                 | 9  | 9      | 0     | 0            | 0       |
| Apocrine carcinoma                  | 19 | 0      | 0     | 3            | 16      |
| Malignant melanoma                  | 28 | 28     | 0     | 0            | 0       |

Absent, negative; mild, focal (1 to 9% of cells); moderate, intermediate (10 to 50%); diffuse (more than 50%).

Table 2: Primer pairs used in RT-PCR.

| Target  | Gene accession | Primer sequence  | AT (°C) | Product size (bp) |
|---------|----------------|------------------|---------|-------------------|
| AQP 3   | NM_004925      | F: 5′-GACAGAAGGAGCTGGTGTCC-3′ | 58      | 199               |
|         |                | R: 5′-AGAGTGACAGCAAGCCAAAAG-3′ |         |                   |
| PGK1    | NM_000291      | F: 5′-GCTGACAAGTTGATGAGAAT-3′ | 58      | 359               |
|         |                | R: 5′-AGGACTTTACCTTCCAGGAGC-3′ |         |                   |

Figure 1: AQP3 mRNA level in skin normal tissue and lesions. RT-PCR showed AQP3 mRNA in normal squamous tissue (NST), solar keratosis (SoK), seborrheic keratosis (ShK), eccrine poroma (EP), sebaceous (SB), squamous cell carcinoma (SCC), Bowen’s disease (BD), and apocrine carcinoma (AC), but AQP3 mRNA was absent in nevocellular nevus (NN), basal cell carcinoma (BC), and malignant melanoma (MM).
situation. Since both basal cell carcinoma and sebaceous carcinoma show negative AQP3 staining, AQP3 is not useful to distinguish basal cell carcinoma with sebaceous differentiation from sebaceous carcinoma. As for sebaceous tumors, which consist of sebaceoma, sebaceous adenoma, and sebaceous carcinoma, their differential diagnosis depends mainly on histological architectural, cytological features, and mitotic rate. Only immunoreactivity of p53 has been reported to act as a useful marker to distinguish between benign and malignant sebaceous carcinomas [22, 23]. In our study, we found the diffuse positivity pattern of AQP3 expressed in all sebaceous hyperplasias and sebaceous adenomas, but not in sebaceous carcinomas. Therefore, AQP3 immunohistochemical staining may have some value for distinguishing benign sebaceous tumors from malignant ones.

It is not surprising that malignant melanoma shows no expression of AQP3 as normal melanocytes within the epidermis do not show AQP3 expression as demonstrated by our prior study [7].
Figure 4: AQP3 expression in malignant skin tumors. Diffuse cytoplasmic membrane staining of AQP3 is identified in solar keratoses (a), Bowen’s disease (b), Paget’s disease (c), squamous cell carcinoma (d), and apocrine carcinoma (e), while negative AQP3 staining is observed in basal cell carcinomas (f), sebaceous carcinomas (g), and malignant melanomas (h) with corresponding figure of HE staining on the lower right-hand corner (magnification: 200x).
5. Conclusion
In summary, our results showed that AQP3 is expressed in most skin tumors except basal cell carcinoma, sebaceous carcinoma, and malignant melanoma, reflecting the biological characteristic of skin tumors. AQP3 has some diagnostic utility in differential diagnosis of skin tumors.

Data Availability
The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest
The authors declare that they have no conflict of interest.

Authors’ Contributions
Dongfeng Niu, Yanhua Bai and Qian Yao contributed equally to this work and are co-first author.

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