Assessing the Expression of Aquaporin 3 Antigen-Recognition Sites in Oral Squamous Cell Carcinoma

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Abstract: Aquaporin 3 (AQP3) serves as a water and glycerol transporter facilitating epithelial cell hydration. Recently, the involvement of AQP3 in cancers has been reported. However, the immunohistochemical expression of AQP3 in carcinomas remains controversial. We hypothesized that differences in aquaporin 3 antigen recognition (AQP3 AR) may influence their expressions. Thus, our study aimed to assess the immunostaining patterns of 3 AQP3 AR sites in oral squamous cell carcinoma (OSCC) and to compare the adjacent areas of high-grade epithelial dysplasia (HG-ED) and normal oral mucosa (NOM). The study group included formalin-fixed OSCC samples (n = 51) with adjacent regions of HG-ED (n = 12) and NOM (n = 51). The tissues were stained with anti-AQP3 antibodies (AR sites at amino acid (AA) 250-C terminus, AA180-228, and N terminus AA1-80) by immunohistochemistry. Our results showed that strong membranous immunostaining was observed for AQP3 AR sites at the AA250-C terminus and AA180-228 in all the samples for NOM and weak AQP3 immunostaining for both the AR sites in all the 12 samples for HG-ED. The invasive front of OSCC samples showed that AQP3 AR at the AA250-C terminus decreased in 42/51 samples (82.4%) and AA180-228 in 47/51 samples (92.2%). Conversely, in the AQP3 AR site at N terminus AA1-80, all samples of the NOM showed negative or slightly positive staining in the cytoplasm of the lower layers. AQP3 expression was increased in 12/12 cases (100%) and 46/51 cases (90.2%) in the HG-ED and invasive front of OSCC, respectively. AQP3 may be used as a biomarker for detecting malignant transformations. AQP3 AR site differences influence their immunohistochemical expression in OSCC.

Key Words: oral cavity, squamous cell carcinoma, epithelial dysplasia, aquaporin 3, immunohistochemistry

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O ral and oropharyngeal cancers together comprise the sixth most common form of cancer in the world. More than 90% of oral cancers are oral squamous cell carcinomas (OSCCs). OSCC is often preceded by oral potentially malignant disorders such as leukoplakia, which is defined as a white plaque of questionable risk, once other known diseases or disorders that carry no increased risk for cancer are ruled out. The presence of epithelial dysplasia in oral potentially malignant disorders is an important prognostic indicator of malignant transformation.

At present, surgery is the preferred treatment for OSCC. However, the 5-year survival rates (28% to 50%) remain unsatisfactory despite progress in the treatment of OSCC over the past few decades.

Aquaporins (AQPs) are water channel proteins that facilitate transepithelial water movement across the cell membrane. In humans, 13 isoforms (AQP0 to AQP12) have been identified. AQPs are categorized as aquaporins.
(AQP0, AQP1, AQP2, AQP4, AQP5, AQP6, and AQP8), which exclusively transport water; aquaglyceroporins (AQP3, AQP7, AQP9, and AQP10), which can transport water, glycerol, and other small molecules; and superaquaporins (AQP11 and AQP12), whose physiological roles remain unclear.6 Previous studies on mice have reported the immunohistochemical expressions and possible roles of AQP3 in carcinomas remains controversial.7,8 Several studies have indicated that overexpression of AQP3 may contribute to tumor cell proliferation in various solid tumors such as gastric adenocarcinoma (GC) and esophageal squamous cell carcinomas (SCCs).14–16 In contrast, growing evidence shows that AQP3 expression decreases in urothelial carcinomas (UCs) and SCCs of the skin, with the molecular mechanism being unclear.17,18 To the best of our knowledge, a few studies have reported the immunohistochemical expressions and possible roles of AQP3 in OSCC, the results of which are controversial.16,19,20 Kusayama et al16 and Ishimoto et al19 used anti-AQP3 antibody prepared from the N terminus AA1-80 peptide of AQP3 in their immunohistochemical studies and reported that AQP3 immunostaining was overexpressed in the OSCC samples, when compared with the normal oral mucosa (NOM) samples. The authors supposed that AQP3 may be involved in the focal adhesion kinase-mitogen-activated protein kinase pathway, which regulates tumor progression and growth in the human OSCC cell lines.16,19 In contrast, in our previous study (2014), we used anti-AQP3 antibody prepared from the AA180-228 peptide of AQP3 in our study and showed that AQP3 immunostaining in OSCC tissues was weaker than that in NOM tissues.20 We suggested that decreased AQP3 expression may be associated with more aggressive tumor behavior and that it increased the incidence of lymphatic metastasis.20 To solve the discrepancy of AQP3 expression in

### Table 1. Clinicopathologic Features of 51 Oral Squamous Cell Carcinoma Samples

| Characteristics        | Cases (%) |
|------------------------|-----------|
| Sex                    |           |
| Male                   | 32 (62.7) |
| Female                 | 19 (37.3) |
| Age                    |           |
| ≥ 65                   | 33 (64.7) |
| < 65                   | 18 (35.3) |
| Location               |           |
| Tongue                 | 38 (74.5) |
| Gingiva                | 5 (9.9)   |
| Floor of the mouth     | 4 (7.8)   |
| Buccal mucosa          | 4 (7.8)   |
| Histologic grade       |           |
| Well                   | 38 (74.5) |
| Moderate to poor       | 13 (25.5) |
| T status               |           |
| T1                     | 28 (54.9) |
| T2+T3                  | 23 (45.1) |
| Lymphatic metastasis   |           |
| Yes                    | 22 (43.1) |
| No                     | 29 (56.9) |

### Table 2. Primary Antibodies of AQP3 Used in this Study

| No. | Recognized Parts of AQP3 | Antibody Host | Clone No. | Dilution | Antigen Retrieval | Incubation | Supplier               |
|-----|--------------------------|---------------|-----------|----------|-------------------|------------|------------------------|
| 1   | AA250-C terminal         | PR            | ab153694  | 1:1000   | CB, 98°C, 40 min  | 4°C, ON    | Abcam                  |
| 2   | AA180-228                | PR            | V214      | 1:100    | Not performed     | RT, 1 h    | Bioworld Technology Inc. |
| 3   | N terminal-AA1-80        | PR            | sc-20811  | 1:100    | CB, 98°C, 40 min  | 4°C, ON    | Santa Cruz             |

AQP3 indicates aquaporin 3; CB, citrate buffer (pH 6.0); ON, overnight; PR, rabbit polyclonal; RT, room temperature.

### Table 3. Expression of AQP3 in the 3 Different AQP3 Antigen Recognitions

| No. Cases (%) | AQP3 Recognition | Score | NOM (N = 51) | HG-ED (N = 12) | OSCC (N = 51) |
|---------------|------------------|-------|--------------|----------------|---------------|
| AA250-C terminal | HM               | 51 (100) | 0 (0) | 43 (84.3) | 9 (17.6) |
| AA180-228      | LM               | 0 (0) | 12 (100) | 8 (15.7) | 42 (82.4) |
| N terminal-AA1-80 | LC            | 51 (100) | 0 (0) | 40 (78.4) | 5 (9.8) |

AQP3 indicates aquaporin 3; HM, high membranous expression, labeling index > 50%; HG-ED, high-grade epithelial dysplasia; HM, high membranous expression, labeling index > 50%; IF, invasive front; LC, low cytoplasmic expression, labeling index ≤ 50%; LM, low membranous expression, labeling index ≤ 50%; NOM, normal oral mucosa; OSCC, oral squamous cell carcinoma; SP, superficial part.
carcinomas, accurate information about aquaporin 3 antigen-recognition (AQP3 AR) sites by anti-AQP3 antibodies is crucial. We hypothesized that differences in AQP3 AR may be indicative of their expression. We investigated the immunostaining patterns of the 3 different AQP3 AR sites in OSCC, comparing the adjacent areas of high-grade (moderate to severe) epithelial dysplasia (HG-ED) and NOM.

MATERIALS AND METHODS

Samples

In total, 51 formalin-fixed, paraffin-embedded biopsy and resection specimens of OSCC, containing simultaneous areas of NOM and/or HG-ED were chosen for this study. The histopathologic diagnoses were confirmed by 2 oral pathologists (N.Y. and K.M.). Clinical data on the patients, such as sex, age, and location, were also included. In addition, pathologic reports were used to assess the histologic grade, T status of the tumors, and lymphatic metastasis (Table 1). Each specimen was categorized as invasive front (IF) of OSCC (n = 51), superficial part (SP) of OSCC (n = 51), NOM (n = 51), and/or HG-ED (n = 12). This study was approved by the Kyushu Dental University Ethics Committee (approved number: 16-8).

Immunohistochemical Study

Between February 2004 and November 2017 at the Department of Oral Pathology, Kyushu Dental University, all the specimens were fixed with 10% formalin and were embedded in paraffin. Four-micrometer-thick sections were stained for AQP3.
deparaffinized in xylene and were serially rehydrated in ethanol. Endogenous peroxidase activity was then quenched with 3% hydrogen peroxide for 20 minutes. For antigen retrieval, if necessary, the sections were heated in 10 mM citrate buffer (pH 6.0) at 98°C for 40 minutes. Nonspecific protein binding was blocked by incubation in 10% normal goat serum for 10 minutes. Thereafter, the specimens were incubated with rabbit polyclonal anti-AQP3 antibodies (AR at AA250-C terminus, AA180-228, and N terminus AA1-80 parts of AQP3) for 1 hour at room temperature or overnight at 4°C. The recognized epitopes and other conditions are summarized in Table 2 and Figure 1B. The tissue sections were then incubated with the secondary antibody for 30 minutes at room temperature. Counterstaining was performed using Mayer's hematoxylin stain for 90 seconds, after which the sections were dehydrated serially in ethanol, cleared with xylene, and mounted on slides with a coverslip.

**Evaluation of Immunohistochemistry**

Localization of staining was recorded, and the labeling index (LI) was calculated by dividing the number of AQP3-positive epithelial cells by the total number of cells, and it was expressed in percentage. Expression of AQP3 localized at the basolateral membranes in the kidney’s collecting duct in normal human tissue microarrays was used as the positive control. For the AQP3 AR sites at AA250-C terminus and AA180-228, the criteria used to define AQP3-positive cells included complete membranous staining. Abnormal staining included absent membranous staining, and cytoplasmic and/or nuclear staining was considered as negative. For the AQP3 AR site at N terminus AA1-80, the epithelial cells were considered as positive when clear cytoplasmic staining was observed. A minimum of 500 cells was counted manually for each study group (NOM, HG-ED, SP, and IF of OSCC). Subsequently, the staining of AR sites at AA250-C terminus and AA180-228 was categorized as high membranous expression (HM: LI > 50%) and low membranous expression (LM: LI ≤ 50%). Staining of AR site at N terminus AA1-80 was categorized as high cytoplasmic expression (HC: LI > 50%) and low cytoplasmic expression (LC: LI ≤ 50%).

**FIGURE 3.** Averages of aquaporin 3 (AQP3) labeling index of the 3 different AQP3 antigen-recognition (AR) sites. The mean labeling index of AQP3 AR at AA250-C terminus (A) and AA180-228 (B) was significantly higher in normal oral mucosa (NOM) than that in high-grade epithelial dysplasia (HG-ED) and invasive front of oral squamous cell carcinoma (IF of OSCC) (P < 0.05). Conversely, the mean labeling index of AQP3 AR at N terminus AA1-80 (C) was significantly higher in HG-ED and IF of OSCC than that in NOM (P < 0.05).
Statistical Analysis

Yate $\chi^2$ test was used to examine the association between AQP3 expression and clinicopathologic information. Mean labeling indices among the study groups were compared using the Mann-Whitney $U$ test. A $P$-value $<0.05$ was considered significant.

RESULTS

Clinical and histopathologic data on the 51 OSCC samples are summarized in Table 1. No correlation between AQP3 expression and clinicopathologic information was observed (data not shown). The overall expression of AQP3 is summarized in Table 3.

Immunostaining of AQP3 AR at AA250-C Terminus

For NOM, all 51 samples showed diffuse, homogenous, and strong immunostaining in the cell membrane, with faint immunostaining in the cytoplasm of cells of the basal, suprabasal, and spinous layers (HM: 100% samples). AQP3 immunostaining was decreased in all the 12 samples of HG-ED (LM: 100% samples). In the SP of OSCC, 43/51 samples retained a considerable membranous expression (HM: 84.3% samples), whereas reduced expression of AQP3 was observed in 42/51 samples in the IF of OSCC (LM: 82.4% samples) (Figs. 2A–C).

The mean LI values of NOM, HG-ED, and IF of OSCC were 84.9 ± 3.1, 5.9 ± 3.9, and 17.4 ± 27.8, respectively. There was a statistically significant decrease in the mean LI of AQP3 AR at the AA250-C terminus in HG-ED and IF of OSCC compared with that of NOM ($P<0.05$) (Fig. 3A).

Immunostaining of AQP3 AR at AA180-228

For NOM, all 51 samples showed diffuse and strong membranous with faint cytoplasmic immunostaining in the suprabasal and spinous cell layers. The basal cells showed trace staining (HM: 100% samples). For HG-ED, SP, and IF of OSCC, AQP3 immunostaining was often
decreased, respectively, for 12/12 samples (LM: 100% samples), 35/51 samples (LM: 68.6% samples), and 47/51 samples (LM: 92.2% samples) (Figs. 2D–F).

The mean LI values for NOM, HG-ED, and IF of OSCC were 82.0 ± 3.7, 2.4 ± 2.6, and 7.3 ± 19.9, respectively. There was a statistically significant decrease in the mean LI of AQP3 AR at AA180-228 in HG-ED and IF of OSCC compared with that of NOM (P < 0.05) (Fig. 3B).

Immunostaining of AQP3 AR at N Terminus AA1-80

For the NOM, all 51/51 samples showed absent or slightly positive staining of the cytoplasm of basal and suprabasal layers (LC: 100% samples). For HG-ED, cytoplasmic AQP3 immunostaining increased to intermediate and upper portions in all 12 samples (HC: 100% samples). In the SP of OSCC, 40/51 samples showed cytoplasmic AQP3 positivity at the periphery of tumor nests, with weaker or almost negative staining in the center (LC: 78.4% samples). More diffuse with moderate to strong cytoplasmic AQP3 immunostaining was observed in 46/51 samples of the IF of OSCC (HC: 90.2% samples) (Figs. 2G–I).

The mean LI values for NOM, HG-ED, and IF of OSCC were 7.4 ± 4.7, 91.1 ± 7.5, and 89.9 ± 17.5, respectively. There was a statistically significant increase in the mean LI of AQP3 AR at N terminus AA1-80 in HG-ED and IF of OSCC compared with that of NOM (P < 0.05) (Fig. 3C).

DISCUSSION

Recently, AQP3 has been reported to be involved in several types of cancers. However, the immunohistochemical expression of AQP3 in carcinomas remains controversial.7,8

FIGURE 5. Schematic illustration of possible results of aquaporin 3 (AQP3) antigen-recognition (AR) site differences in high-grade epithelial dysplasia (HG-ED) and oral squamous cell carcinoma (OSCC). The dysplastic and tumor cells might produce a lot of nascent AQP3 protein. AA135-157, AA180-228, and AA250-C terminus parts of the nascent AQP3 protein might be degraded (described later), whereas N terminus AA1-80 part of the nascent AQP3 protein was retained and could be detected by anti-AQP3 antibody prepared from N terminus AA1-80 peptide of AQP3. Consequently, in the AQP3 AR site at N terminus AA1-80, cytoplasmic AQP3 immunostaining increased in the HG-ED and invasive front of OSCC. In contrast, membrane-type 1 matrix metalloproteinase and other proteases, which were secreted from the tumor cells, might bind the disordered regions (AA135-157, AA182-188, AA208-218, and AA269-276) of both mature (membranous) and nascent AQP3 proteins, resulting in degradation of these disordered regions and surrounding peptides. Thus, in the AQP3 AR sites at AA180-228 and AA250-C terminus, AQP3 immunostaining was decreased in HG-ED and invasive front of OSCC. Moreover, degradation of the disordered protein-asparagine-proline-alanine (NPA) site (AA215-217) may impair water movement across cell membranes and cause water retention around the dysplastic epithelium, which might result in discohesion and migration of the cancer cells.
Differences in AQP3 AR sites may influence the immunohistochemical expression patterns. To our knowledge, this is the first attempt to evaluate the immunostaining patterns of 3 different AQP3 AR sites in NOM, HG-ED, SP, and IF of OSCC, which would improve our understanding of the role of AQP3 in oral carcinogenesis.

**AQP3 AR Site at N Terminus AA1-80**

In AQP3 AR site at N terminus AA1-80, NOM stained negative or slightly positive in the cytoplasm of basal and suprabasal layers. Normally, in human and rat tissues, AQP3 was clearly expressed in the cell membranes of the squamous epithelia in the skin and oral mucosa.13,21 It is probable that plenty of mature (membranous) AQP3s in the NOM may not be recognized by anti-AQP3 antibody prepared from the N terminus AA1-80 peptide of AQP3, while this antibody may recognize nascent AQP3 protein, which was slightly produced in the NOM (Fig. 4). Cytoplasmic AQP3 immunostaining was increased in HG-ED and IF of OSCC. It is possible that the dysplastic and tumor cells might generate a lot of nascent AQP3 protein. AA180-228 and AA250-C terminus regions of the nascent AQP3 protein might be degraded (described later), whereas the N terminus AA1-80 part of the nascent AQP3 protein was retained and could be detected by anti-AQP3 antibody prepared from N terminus AA1-80 peptide of AQP3 (Fig. 5).

Our results were in agreement with previous studies using anti-AQP3 antibody prepared from N terminus AA1-80 peptide of AQP3 in the dysplastic squamous epithelium of the cervix, SCC of the cervix, esophagus, and oral cavity, and GCs (Table 4).14–16,19,22,23 Increased N terminus AA1-80 part of nascent AQP3 protein in GC is associated with an increase in nuclear translocation of β-catenin, which leads to the proliferation of tumor cells.23 In addition, overexpression of N terminus AA1-80 part of nascent AQP3 protein correlates with downregulation of E-cadherin and overexpression of vimentin in poorly differentiated GC, thereby suggesting a role of AQP3 in the epithelial-to-mesenchymal transition process.15 Moreover, in OSCC, overexpression of N terminus AA1-80 part of nascent AQP3 protein may be related to the focal adhesion kinase-mitogen-activated protein kinase signaling pathway, resulting in tumor cell proliferation.16,19

**AQP3 AR Sites at AA180-228 and AA250-C Terminus**

In AQP3 AR sites at AA180-228 and AA250-C terminus, AQP3 staining was strong in the cell membrane, but it was faint in the cytoplasm of the NOM, which is consistent with the fact that AQP3 is a transmembrane protein.13,21,24 A large amount of mature (membranous) AQP3 with a small amount of nascent AQP3 protein in the NOM may be recognized by anti-AQP3 antibody prepared from AA180-228 and AA250-C terminus peptide of AQP3 (Fig. 4). AQP3 immunostaining was decreased in HG-ED and IF of OSCC. It is widely accepted that tumor cells secrete proteases that can degrade the tumor barriers and thus facilitate tumor progression and invasion.25 Membrane-type 1 matrix metalloproteinase is one of the proteases that degrade extracellular matrix proteins.

**TABLE 4. Summary of AQP3 Antigen Recognitions at N Terminus AA1–80 Expression Patterns in the Reported Carcinomas**

| No. | Recognized Part of AQP3 | Organ | Antibody | Host | Expression Pattern Type | Expression Pattern Type Expression Pattern References |
|-----|------------------------|-------|----------|------|-------------------------|----------------------------------------------------|
| 1   | N terminal             | Stomach | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 2   | N terminal             | Esophagus | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 3   | N terminal             | Oral cavity | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 4   | N terminal             | Cervix | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 5   | N terminal             | Stomach | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 6   | N terminal             | Oral cavity | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 7   | N terminal             | Cervix | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| 8   | N terminal             | Stomach | PR      | Rabbit polyclonal | Increased CP | Increased CP, SCC, gastric mucosa, invasive front; NP, not performed; PR, rabbit polyclonal; SCC, squamous cell carcinoma. |
| No. | Recognized Part of AQP3 | Antibody Host | Organ | Type | Expression Pattern | Expression Pattern | Type | Expression Pattern | References |
|-----|-------------------------|---------------|-------|------|-------------------|-------------------|------|-------------------|------------|
| 1   | C terminal              | PR            | Lung  | BSE  | MB                | NC                | SCC  | 62% of cases showed loss of MB | Liu et al 29 |
| 2   | C terminal              | PR            | Lung  | BSE  | MB                | NC                | BAC with invasive ADC SCC | Machida et al 30 |
| 3   | C terminal              | PR            | Skin  | Squamous epithelium | MB | NC | 100% of cases showed loss of MB | Voss et al 18 |
| 4   | C terminal              | PG            | Urinary bladder | Urothelium | MB | NC | 100% of cases showed loss of MB | Rubenwolf and colleagues 31,32 |
| 5   | C terminal              | PR            | Urinary bladder | Urothelium | MB | NC | 41% of cases showed loss of MB | Otto et al 17 |
| 6   | C terminal              | PR            | Thyroid gland | C cells and follicular cells | Almost negative | NP | 90% of cases showed increased MB | Niu et al 33 |
| 7   | C terminal              | PG            | Urinary bladder | Urothelium | MB | NC | 67% of cases showed loss of MB | Rubenwolf et al 34 |
| 8   | AA180-228              | PR            | Oral cavity | Squamous epithelium | MB | Loss of MB | SCC | Generally the loss of MB | Matsuo and Kawano 30 |
| 9   | C terminal              | PR            | Skin  | Squamous epithelium | MB | NC | SCC | Generally the loss of MB | Seleti et al 35 |
| 10  | C terminal              | PR            | Liver | Hepatocyte | Almost negative | NP | 93% of cases showed increased MB | Peng et al 36 |
| 11  | C terminal              | PR            | Pancreas | Ductal cells | Urothelium | Almost negative | NP | PDA | 100% of cases showed loss of MB | Direito et al 37,38 |
| 12  | C terminal              | PR            | Prostate gland | Glandular epithelial cells | Almost negative | NP | High-risk PC | Generally the loss of MB | Brundl et al 39 |
| 13  | C terminal              | PR            | Breast | Ductal cells | MB | NC | TNBC | 61% of cases showed increased MB | Zhu et al 40 |
| 14  | C terminal              | PR            | Oral cavity | Squamous epithelium | MB | Loss of MB | SCC | 84% of cases of the IF part showed loss of MB | This study |
| 15  | C terminal              | PR            | Oral cavity | Squamous epithelium | MB | Loss of MB | SCC | 92% of cases of the IF part showed loss of MB | This study |

AA indicates amino acid; ADC, pulmonary adenocarcinoma; AQP3, aquaporin 3; BAC, bronchioloalveolar carcinoma; BSE, bronchial surface epithelium; HCC, hepatocellular carcinoma; IF, invasive front; MB, membranous expression; MTC, medullary thyroid carcinoma; NP, not performed; PC, prostate adenocarcinoma; PDA, pancreatic ductal adenocarcinoma; PG, goat polyclonal; PR, rabbit polyclonal; pT1 UC, urothelial carcinoma invades connective tissue; pT2 UC, urothelial carcinoma invades muscle; SCC, squamous cell carcinoma; TNBC, triple-negative breast cancer.
Assessing the Expression of AQP3 AR Sites in OSCC

CONCLUSIONS

To summarize, AQP3 could be used as a novel biomarker for detecting malignant transformations in the squamous epithelium. Our findings show that the differences in AQP3 AR sites affect their immunohistochemical expression in OSCC. In the AQP3 AR sites at C terminus AA180, AQP3 immunostaining was found to be increased in the dysplastic squamous epithelium compared with the normal squamous epithelium, whereas in AQP3 AR sites at AA180-228 and AA250-C terminus, AQP3 expression was weaker and reduced in the dysplastic squamous epithelium than that in the normal squamous epithelium. Our data suggest that understanding the AQP3 AR site of each anti-AQP3 antibody before performing an immunohistochemistry analysis is critical. A combination expression pattern of N terminus and C terminus parts of AQP3 might be a more accurate marker for detecting malignant transformation. However, it is possible that, on the basis of the field of carcinogenesis, the areas adjacent to carcinoma already harbor mutations that may not yet cause phenotypical features. Further studies, with the use of additional molecular biology, are warranted to confirm our results and precisely investigate the molecular mechanism underlying the role of AQP3 in carcinogenesis.

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