Expression of Plectin-1 and Trichohyalin in Human Tongue Cancer Cells

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Abstract

In basal squamous cells, plectin-1 interacts with intermediate filaments, whereas trichohyalin, which is distributed primarily in the medulla and inner root sheath cells of human hair follicles, plays a role in strengthening cells during keratinization. Although both cytoskeletal proteins occur in trace amounts in human tongue epithelial cells, there are minimal data on their expression in human tongue primary cancer cells. We therefore investigated the expression of plectin-1 and trichohyalin in human tongue cancer cells. Open Journal of Stomatology, 8, 196-204. https://doi.org/10.4236/ojst.2018.86019

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1. Introduction

Cancer cells are characterized by phenotypic and biological heterogeneity. Similar to epithelial-to-mesenchymal transition (EMT), the ability of cancer cells to infiltrate tissues and metastasize during carcinogenesis is associated with changes in the expression of cell structural and adhesion molecules as well as matrix metalloproteases [1] [2] [3]. Accordingly, investigations into the changes in expression of these molecules would be useful in developing diagnostic methods and determining prognosis.

Proteins of the plakin family play important roles in ensuring the integrity of epithelial, muscle, and nerve cells [4]. Plectin-1, which is 500-kDa cross-linker protein, belongs to the plakin family of cytoskeletal proteins and interacts with intermediate filaments, being localized into cells in some tissues. As plectin-1 functions as the binding protein of hemi-desmosome bullous pemphigoid antigen [2] and links to intermediate filaments, it was hypothesized that plectin-1 contributes to cell mobility and cytoskeleton formation [5] [6] [7]. However, details regarding the kinetics of plectin-1 in human oral tissues have not been reported.

Trichohyalin is a protein of approximately 200 kDa that is distributed primarily in the medulla and inner root sheath cells of human hair follicles, and it is believed to play a role in maintaining cell strength during keratinization [8] [9]. Although there is no information on its expression in human oral primary cancer cells, trichohyalin expression has been reported in tongue papilla in normal human oral tissue [10] [11].

As plectin-1 and trichohyalin interact with intermediate filaments or actin filaments, it is reasonable to hypothesize that they play roles in the formation of invadopodia and/or filopodia. However, there is little information available regarding their expression in human tongue primary cancer cells.

In the present study, we investigated the expression of plectin-1 and trichohyalin in DOK human tongue epithelial cells [12] and BICR31 tongue cancer cells [13] using western blotting and FITC-labeled immunocytochemistry techniques.
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humidified atmosphere containing 5% CO₂ in air at 37˚C.

2.2. Western Blot Analyses

DOK and BICR31 cells were cultivated in 25 cm² tissue culture flasks and then lysed. The cell lysates were solubilized in SDS-PAGE sample buffer, and protein concentration was determined using the bicinchoninic acid method [14]. Equal amounts of protein samples were fractionated and then transferred electrophoretically onto PVDF membranes [15]. After blocking with 1% bovine serum albumin (BSA) at 4˚C for overnight and incubation with antibodies against plectin-1 or trichohyalin (Table 1) at 4˚C for overnight, the membranes were labeled using the streptavidin-biotin method [16]. Protein bands were then visualized by subsequent exposure of the membranes on X-ray film, with the molecular size of proteins, specificity of antibodies, and expression of each protein confirmed by western blot analysis.

2.3. Immunocytochemical Analyses

DOK and BICR31 cells were seeded at a density of 1000 cells/well in chamber slides and incubated in DMEM, as previously described. After cells were fixed with cold methanol for 20 min, non-specific reactions were blocked with phosphate-buffered saline (PBS) containing 1% BSA. Treated cells were incubated with the previously described antibodies (Table 1), followed by RITC-conjugated anti-immunoglobulins (DAKO A/S) for cytokeratins or FITC-conjugated anti-immunoglobulins (DAKO A/S) for plectin-1 and trichohyalin. All fluorescence images were finally observed using an all-in-one fluorescent microscopy system (BZ-9000; Keyence Japan, Osaka, Japan). Samples incubated with PBS instead of primary antibody were used as negative controls.

Table 1. The list of antibodies.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Cytology</th>
<th>Blotting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plectin 1</td>
<td>C-20</td>
<td>1:200</td>
<td>1:400</td>
</tr>
<tr>
<td>Trichohyalin</td>
<td>K-16</td>
<td>1:200</td>
<td>1:400</td>
</tr>
<tr>
<td>Cytokeratin 1/10</td>
<td>LH1</td>
<td>1:100</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 1. Phase contrast microscopy images (×40, bar = 125 µm) of experimental cells.
3. Results

3.1. Western Blot Profiles

Western blot analyses confirmed the molecular weight and expression of each protein (Figure 2). Anti-plectin-1 antibody reacted in the extreme high-molecular-weight region with BICR31 cells (Figure 2, arrow) but not DOK cells. Both DOK and BICR31 cells expressed trichohyalin, which showed a 200-kDa band.

3.2. Immunocytochemical Findings

On immunocytochemical staining, plectin-1 (Figure 3) and trichohyalin (Figure 4) were localized. There were correlations between western blot and immunocytoLOGY results for plectin-1. No localization of plectin-1 was observed in most DOK cells, but strong immunoreaction was detected in BICR31 cells. Additionally, plectin-1 and CK 1/10 colocalized widely in BICR31 cells (Figure 3, merge). Conversely, no immunocytochemical correlations were observed for trichohyalin. Anti-trichohyalin antibody reacted in the cytoplasm and partially in the nuclear region (Figure 4). Trichohyalin was diffusely distributed in DOK cells, and the immunoreaction was weak. The anti-trichohyalin antibody reacted stronger in BICR31 cells than DOK cells.

4. Discussion

Plectin-1 was the first cytoskeletal component discovered to connect intermediate filaments in epithelial cells of the small intestine, liver, and urinary bladder. In addition, plectin-1 is expressed in muscle and nerve cells, and it is hypothesized that the protein is involved in complex functions [4] [17] because of its distribution in crosslinks of microtubules and actin filaments. It is generally known that plectin-1 is not expressed in normal tongue cells [17], and we observed almost no plectin-1 expression in DOK cells, a human tongue epithelial cell line. Therefore, it is assumed that tongue epithelial cells have a “thin” cell structure and that the cytoskeleton is maintained by an intercellular component. Plectin-1 knock down in human liver cells treated with plectin-1 siRNA was shown to induce a decrease in the expression of CK18, the disorganization of intermediate filaments, and an increase in actin fibers [18]. Accordingly, chief cytoskeleton components in human tongue epithelial cells could be other molecules such as actin, tubulin, and heat shock proteins [19]. By contrast, BICR31 human tongue cancer cells showed clear expression of plectin-1. Similarly, negative immunoreaction showed that plectin-1 is not expressed in normal human pancreas cells, but an increase in plectin-1 expression was reported during pancreatic ductal carcinogenesis and metastasis to the lymph nodes, liver, and peritoneum [20]. These data suggest that plectin-1 could serve as a tumor marker in tongue and pancreatic tissues. Additionally, the observation that ablation of plectin-1 in human colon carcinoma cells treated with siRNA impairs their growth, migration, and adhesion [21] suggests that plectin-1 is involved in malignant transformation of epithelial cells.
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Figure 2. Western blotting analyses. BICR31 cells showed high expression of plectin-1 (arrow). No differences between DOK and BICR31 cells were observed in terms of trichohyalin expression (arrowhead).

Figure 3. Immunocytochemical observations of plectin-1 expression in experimental cells (×200, bar = 25 μm). Although almost no plectin-1 expression was observed in DOK cells, BICR31 cells clearly expressed plectin-1.
Immunocytological observations of trichohyalin expression in experimental cells (×200, bar = 25 µm). In immunocytological staining, trace or diffuse localization of trichohyalin was observed in all compartments of DOK cells. However, trichohyalin expression was widely distributed in BICR31 cells, and some colocalization of trichohyalin and CK 1/10 was observed (white arrowhead in merged image).

Immunoelectron microscopy studies recently revealed that trichohyalin, which localizes near parallel bundles of intermediate filaments in a manner that promotes the aggregation and lateral alignment of filament bundles [22], exhibits up-regulated expression in human tongue epithelia [23] and skin epidermal diseases, including squamous cell carcinoma [24]. In the present study, expression of trichohyalin was not related to the malignancy of cells and did not change over succeeding generations of cells.

Plectin-1 and trichohyalin are known to connect intermediate filaments of intact epithelial cells [4] [22]. However, there is little information regarding the mode of their interaction in human tongue primary cancer cells. Although we performed co-immunoprecipitation analyses for CK 1/10 followed by western blot analyses, we did not obtain clear, reproducible results in immunoreactions.
for plectin-1 and trichohyalin (data not shown). A recent study using a sensitive fluorescent protein-binding assay showed that the C-terminus of plectin-1 interacts predominantly with dimeric CK 1/10 or CK 5/14 rather than monomeric cytokeratins [25]. Accordingly, the results of segmentation analyses might show that plectin-1 or trichohyalin and CK do not interact in the presence of non-ionic surfactants and that trichohyalin and CK 1/10 do not form chemical bonds.

The results of the present study indicate that trichohyalin is expressed by cancerous tongue epithelial cells during various stages of malignancy and that plectin-1 is an index of malignancy.

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