Coexpression of cytokeratins, involucrin, and blood group antigens in oral squamous cell carcinomas

Patrick D. Toto, DDS, MS, and Hasan Nadimi, DMS, MS, Maywood, Ill.

LOYOLA UNIVERSITY OF CHICAGO SCHOOL OF DENTISTRY

The well and poorly differentiated oral squamous carcinomas preferentially express proteins, blood group antigens, and contain associated dendritic Langerhans' cells. Keratin pearls in well-differentiated carcinomas simulate the differentiation pathway of the normal oral squamous epithelium, whereas poorly differentiated carcinomas do not and appear more heterogeneous. Terminally keratinized cells correlate with involucrin and expression of blood group antigens in keratin pearls, a feature that differs from the nonkeratinizing normal epithelium in which such carcinomas arise. Dendritic Langerhans' cells are reduced in number in squamous carcinomas.

Immunohistochemical and biochemical studies of normal oral stratified squamous epithelium have demonstrated similarities and differences in keratinizing and nonkeratinizing regional expression of keratin proteins, dependent on the degree of maturation. The degree of maturation appears to correlate with the expression by squamous cells of involucrin, carcinoembryonic antigen (CEA), blood group antigens A, B, and H type, HLA-ABC, beta 2-microglobulin (β2-M), and dendritic Langerhans' cells (DLCs). DLCs are found in greater numbers in nonkeratinizing than keratinizing mucosa, indicative of the effect of regional distribution in oral epithelium, which is inversely related to the degree of keratinization.

Immunohistochemical studies of oral squamous carcinomas have shown cellular heterogeneity in the expression of keratin proteins dependent on the degree of differentiation of the lesion. Involucrin is expressed in cells that terminally differentiate in well-differentiated (WD) carcinoma; however, in the intermediate or poorly differentiated (PD) carcinomas, it may appear as a mosaic of squamous cells, or be absent. CEA is expressed but blood group antigens A and B are not expressed in squamous carcinomas; however, their precursor, blood group antigen H type 2, is expressed and may accumulate in the cytoplasm of squamous cells. HLA-ABC and β2-M, reportedly, are not expressed in squamous carcinoma. DLCs are found in greater numbers in oral carcinoma than in normal epithelium. In squamous cell carcinoma of skin, DLCs may be increased, equivalent, or decreased in number. DLCs are adendritic in cervical epithelial metaplasia. Laminin and fibronectin are glycoproteins that constitute the basal lamina of squamous epithelium, and may be enzymically degraded in carcinoma.

In this report, we show that selected proteins, blood group antigens, and associated DLCs are expressed with nucleated cells in keratin pearls of WD carcinomas, which simulate the normal epithelium. On the other hand, in PD carcinoma there is greater variability in such expression, consistent with cellular heterogeneity, alteration, and apparent arrest in stage differentiation of stratified squamous epithelium.

MATERIAL AND METHODS

Thirty-six cases of well-differentiated (tongue 15, soft palate 6, buccal mucosa 3, and floor of the mouth 12) and 24 of poorly differentiated (tongue 10, soft palate 2, buccal mucosa 4, and floor of the mouth 8) squamous carcinomas and, for control, 10 specimens of normal human oral mucosa (ventral tongue surface 3, soft palate 2, floor of the mouth 3, buccal mucosa 1, and gingiva 1) surgically excised for other treat-
Table I.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Source</th>
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<tbody>
<tr>
<td>IgG fraction</td>
<td>Keratin 48 kD to 67 kD</td>
<td>Rabbit</td>
</tr>
<tr>
<td>IgG fraction</td>
<td>Keratin 42 kD to 67 kD</td>
<td>Rabbit</td>
</tr>
<tr>
<td>IgG fraction</td>
<td>Involucrin 68 kD</td>
<td>Rabbit</td>
</tr>
<tr>
<td>IgG2 fraction</td>
<td>Human lymphocyte antigen, HLA-ABC</td>
<td>Mouse</td>
</tr>
<tr>
<td>IgG fraction</td>
<td>Carcinoembryonic antigen, CEA</td>
<td>Rabbit</td>
</tr>
<tr>
<td>IgM fraction</td>
<td>Blood group antigen type A</td>
<td>Mouse</td>
</tr>
<tr>
<td>IgM fraction</td>
<td>Blood group antigen type B</td>
<td>Mouse</td>
</tr>
<tr>
<td>IgM fraction</td>
<td>Blood group antigen H type 2</td>
<td>Mouse</td>
</tr>
<tr>
<td>Whole antiserum</td>
<td>Laminin</td>
<td>Rabbit</td>
</tr>
<tr>
<td>IgG fraction</td>
<td>Bovine S-100 (brain)</td>
<td>Rabbit</td>
</tr>
</tbody>
</table>

Table II. Distribution of proteins in membrane (M) and cytoplasm (C) in epithelium of squamous carcinoma

<table>
<thead>
<tr>
<th></th>
<th>WD</th>
<th>PD</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>C</td>
<td>M</td>
<td>C</td>
</tr>
<tr>
<td>Keratin</td>
<td>+</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Involucrin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CEA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>B2-M</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

+, Partial absence of stain in membrane and partial presence in cytoplasm of cells.

RESULTS

In normal epithelium and in WD carcinomas, keratin proteins were stained in the cytoplasm and on cell membranes at intercellular bridges, except for dissociated cells and squamous cells in PD carcinoma (Table II). Cells that stained with antibody to 48 to 67 kD keratins were localized in the upper stratum spinosum of normal epithelium, the nucleated cells but not basaloid cells in keratin pearls of WD carcinoma, and absent from or variably stained intensities in scattered cells of PD carcinomas. Antibody to 42 to 67 kD keratins in normal epithelium identified keratin proteins that stained with apparent increasing intensity from the basal cell layer to surface cells. This staining pattern was simulated in the nucleated cells of keratin pearls observed from basaloid cells to those adjacent to the centrally located and terminally keratinized
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Fig. 1. Intracellular localization of total squamous cell keratin in well-differentiated carcinoma. (PAP. Original magnification, ×400.)

Fig. 2. Cell membrane and intercellular localization of CEA in well differentiated squamous carcinoma. (PAP. Original magnification, ×250.)

Fig. 3. Involucrin localization in cell membrane in poorly differentiated carcinoma. Few scattered cells are reactive (arrow). (PAP. Original magnification, ×250.)

Fig. 4. Cell membrane localization of β-2M in well-differentiated carcinoma (arrow). (PAP. Original magnification, ×250.)

In the upper stratum spinosum of normal epithelium, the stain for involucrin was localized to the cytoplasm and membrane, whereas that for CEA was localized only to the cell membrane. In WD carcinomas, involucrin and CEA were expressed in the cytoplasm and on the cell membrane of nucleated cells, but not in basaloid cells (Fig. 2). In PD carcinomas, involucrin reacted with cytoplasm of only a few scattered cells (Fig. 3). CEA was reactive with both cytoplasm and cell membrane.

In normal epithelium, HLA-ABC and β2-M were localized to the cell membrane. In squamous carcinomas, HLA-ABC was unreactive; however, β2-M was localized to both cell membrane and cytoplasm of nucleated cells in WD carcinomas (Fig. 1). The cells in PD carcinoma were weakly reactive and of variable staining intensities with antibody to total squamous epithelium.

In normal epithelium, HLA-ABC and β2-M were localized to the cell membrane. In squamous carcinomas, HLA-ABC was unreactive; however, β2-M was localized to both cell membrane and cytoplasm of nucleated cells in WD carcinomas, and, except for dissociated cells, in PD carcinomas (Fig. 4).

The distribution of DLCs in normal epithelium differed significantly from WD and PD carcinomas (Table III). The range, mean, and standard error of the mean/HPF were 16-28/HPF, 19.2 ± 2.1 in normal epithelium, 5-16/HPF, 9.3 ± 2.3 in WD carci-

Table III. Langerhans' cells in epithelium (HPF × 250) per 1000 cells

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean</th>
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</thead>
<tbody>
<tr>
<td>Normal (10)</td>
<td>16-28</td>
<td>19.2 ± 2.1</td>
</tr>
<tr>
<td>WD carcinoma (36) (keratin pearls)</td>
<td>5-16</td>
<td>9.3 ± 2.3</td>
</tr>
<tr>
<td>PD carcinoma (24)</td>
<td>0-5</td>
<td>2.2 ± 2.0</td>
</tr>
</tbody>
</table>

p < 0.01
Fig. 5. Localization of S-100 positive dendritic DLCs in well-differentiated carcinoma. (PAP. Original magnification, x400.)

Fig. 6. Basement membrane localization of laminin in well-differentiated carcinoma. (PAP. Original magnification, x250.)

Table IV. Blood group antigens in epithelium

<table>
<thead>
<tr>
<th></th>
<th>A1/A2</th>
<th>B</th>
<th>H2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (10)</td>
<td>4/10</td>
<td>1/10</td>
<td>10/10</td>
</tr>
<tr>
<td>WD carcinoma (10) (keratin pearls)</td>
<td>1/10</td>
<td>1/10</td>
<td>9/10</td>
</tr>
<tr>
<td>PD carcinoma (10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

of nucleated cells in keratin pearls, A1A2, B, and H type 2. PD carcinoma cells were essentially unreactive for blood group antigens, inasmuch as only a few cells were weakly stained for blood group II type 2 antigen.

In both normal epithelium and squamous carcinomas, laminin and fibronectin were stained in a linear pattern at the interface zone, except for discontinuities observed in both WD and PD carcinomas (Fig. 6). Stromal capillaries were clearly reactive for both proteins.

DISCUSSION

Well-differentiated and poorly differentiated carcinomas may arise in both keratinizing and nonkeratinizing regions of oral mucosa, which are known to produce distinct combinations of keratin proteins in their differentiation pathways. However, the cell collectives that form keratin pearls in WD carcinomas simulate a differentiation pathway of normal epithelium, and produce high molecular weight keratin proteins among terminally keratinized cells that simulate the normal epithelium. In contrast, PD carcinomas form cell collectives arranged in sheets, cords, and individual islets that variably express high molecular weight keratin proteins (48 to 67 kd and 42 to 67 kd) as detected with polyclonal antibodies. This variability of keratin protein staining intensities of the cells in PD carcinomas suggests a heterogeneity of cell differentiation.

The apparent difference in keratin protein staining patterns between the cells of WD and PD carcinomas also correlates with the expression of involucrin. Involucrin, a 68 kd protein, is only expressed in the spinous layer of normal epithelial cells that terminally keratinize. The nucleated cells in keratin pearls express involucrin in spinous cells but not in nucleated basaloid cells of WD carcinoma, a feature that simulates normal epithelium. In contrast, only a few scattered cells express involucrin in PD carcinomas. Moreover, in normal epithelium, involucrin is first expressed as a soluble protein in lower spinous cell layer and is catalyzed by gamma-transglutaminase to gamma-glutamyllysine, which forms cross-linked insoluble membrane involucrin in upper spinous cells that terminally keratinize. This indi-
cates that failure of cell maturation occurs in PD carcinoma inasmuch as only few cells enter a terminal keratin protein differentiative pathway.

The notion that keratin pearls in WD carcinomas simulate the normal stratified squamous epithelium differentiation pathway may be supported by the coexpression of blood group antigens A1-A2, B, and H type 2. The blood group antigen H type 2 is the precursor molecule of blood groups A and B, which are localized only in spinous cells of normal epithelium, and in the nucleated cells but not basloid cells of keratin pearls in WD carcinoma. In contrast, only a few scattered weakly stained spinous cells express antigen H type 2 in PD carcinoma.20, 21

In a similar staining pattern, CEA and β2-M but not HLA-ABC are coexpressed on the surfaces of cells and cytoplasm in keratin pearls of WD carcinoma. In PD carcinomas, CEA and β2-M are expressed in the cytoplasm and in scattered cells only on the surface membrane that indicates a tendency toward cytoplasmic accumulation of these proteins. Although β2-M is expressed in both WD and PD carcinomas, HLA-ABC is not expressed in cells of such neoplasms.22, 23 HLA-ABC is reactive in normal epithelium with the use of monoclonal antibodies to stain sections of formalin-fixed paraffin-embedded specimens. It is possible that under similar conditions HLA-ABC molecules may be expressed in low concentration or lost in squamous carcinomas. β2-M and HLA-ABC are transcribed on different chromosomes. It is possible that β2-M is first transcribed but HLA-ABC heavy-chain is not in squamous carcinomas. Melanocytes are not evident in WD or PD carcinomas, either by S-100 or Fontana-Masson staining methods, consistent with their reported absence in hyperproliferative epidermal disorders.24

DLCs are reduced in number in WD and in PD carcinoma. They also manifest cytomorphic changes characterized by reduced number of dendrites, and may appear adendritic in PD carcinoma, as previously reported.28 In contrast, a well-documented study shows an increase in number of DLCs in oral squamous carcinoma above that of normal epithelium.29 In skin, DLCs reportedly are increased,25 equivalent,26 or decreased27 in number. Although the DLCs are associated with spinous cell differentiation in squamous epithium, it is not clear why there are apparent differences reported in squamous carcinoma.

Laminin and fibronectin both are expressed in the basement membrane in normal epithelium. In WD and PD carcinomas, the observed discontinuities in laminin and fibronectin may be attributed to enzymic degradation of the basement membrane that facilitates detachment and migration of cells in stroma.29, 30

REFERENCES

Reprint requests to:
Dr. Patrick D. Toto
Loyola University of Chicago
School of Dentistry
2160 South First Ave.
Maywood, IL 60153