Markers shaping the future of preventive dentistry

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Abstract
Detecting pathologies at their earliest stages can significantly affect patient discomfort, prognosis, therapeutic intervention, survival rates, and recurrence. The use of tissue biomarkers in basic and clinical research and even in clinical practice not only became very common but also their presence as primary endpoints in clinical trials are now very well accepted. The use of tissue biomarkers, and mainly laboratory-measured biomarkers, in clinical set up is somewhat newer, and the suitable approaches to this practice are still in the process of development and refinement. This review highlights contemporary innovations and explores recent discoveries about markers in lesions of oral cavity.

Keywords
Antigen, intracellular, oral lesions, tissue markers

Introduction
Markers usage in premalignant and malignant lesions of oral cavity has a great potential of early detection and diagnosis of oral cancer. Immunohistochemistry (IHC) could be used as a great tool as an effective and advanced marker. Immunohistochemistry method consists of improvements of immunofluorescence, immunoperoxidase, and avidin-biotin complex technique. This allows application of certain tests to paraffin sections of fixed tissues and the production of highly specific antibodies, especially monoclonal antibodies. The drawbacks of this marker tool could be the reliability of the given test is well established and validity with control of results as multiple researchers with diverse skills and knowledge are interpreting the results.

IHC
Tissue markers examined by IHC in precancerous and cancerous lesions:

Cell surface markers
- Carbohydrates
- Blood group antigens A + B + H
- Blood group antigen precursors
- Squamous carcinoma antigens - Ca1, TA - 4
- SQM - 1 and 3H - 1

Intra cellular markers
- Cytokeratins
- Filaggrin
- Involucrin
- Desmosomal proteins
- Carcinoma antigens 17, 13
- Quantitative DNA
  - Silver-binding nucleolar organizing regions
- Oncogenes
- Arachidonic acid products
- Gamma-glutamyl transpeptidase
- Lactate dehydrogenase
- Guanidinobenzoatase.

Basement membrane markers
- Laminin
- Type 1V collagen
- Fibronectin.

Matrix markers
- Procollagen
- Tenascin
- Immunocompetent cells
- Immunoglobulins.

The epithelial surface antigens of squamous epithelium and their reaction patterns and limitations of the methods are given in Table 1.[3,4] The intracellular antigenic constituents and products of squamous epithelium and their reaction patterns and limitations of the methods are given in Table 2.[5,6] The antigenic constituents of the basement membrane zone and subepithelial tissue and their reaction patterns and limitations of the methods are given in Table 3.[7,8]

**Stromal changes**

- The local stromal reaction has been the subject of much speculation, particularly with regard to its role in immunological defense reactions against precancerous and cancerous lesions.
- IHC and electron microscopy made possible to study details of changes in cellular infiltrates within the epithelium and the connective tissue.

**Procollagen**

1. A marked increase in procollagen could be demonstrated in the stromal portion of malignant epidermal lesions suggesting increased turnover.

**Immunocompetent cells**

1. An overall increase in the number of immunocompetent cells correlating with the degree of dysplasia and differentiation of carcinomas was found.
2. Lymphocytes, predominantly T-cells (OKT8/Leu-2a-positive, corresponding to suppressor/cytotoxic cells) appears in the epithelial/tumoral components and in the adjacent stroma.

**Table 1:** Reaction pattern and limitations of the methods of epithelial surface antigens of squamous epithelium

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal mucosa</th>
<th>Dysplasia</th>
<th>Carcinoma</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood group antigens</td>
<td>Cell surface of cell above basal cells</td>
<td>Loss and patchy distribution</td>
<td>Loss and patchy distribution</td>
<td>Individual variations&lt;br&gt;• Loss in:&lt;br&gt;• Wound healing&lt;br&gt;• Cellular locomotion&lt;br&gt;• Erosive LP&lt;br&gt;• Irradiation&lt;br&gt;• False-negative results&lt;br&gt;• False-positive results</td>
</tr>
<tr>
<td>Blood group antigen precursors</td>
<td>Cell surface of basal and suprabasal cells</td>
<td>Accumulation in all cell layers</td>
<td>Irregular distribution</td>
<td>Individual variations&lt;br&gt;Not yet sufficiently studied</td>
</tr>
<tr>
<td>Lectin binding</td>
<td>Cell surface of all epithelial layers</td>
<td>Loss of binding with increase in severity of dysplasia</td>
<td>Loss of binding</td>
<td>Partial loss and changes in wound healing and cellular locomotion</td>
</tr>
<tr>
<td>Ca antigens (glycoprotein) - Not specific</td>
<td>Negative</td>
<td>Variation</td>
<td>Positive staining in majority of cases</td>
<td>Negative reactions in carcinomas&lt;br&gt;Positive reactions in inflammatory lesions (Fibrous epulis, pyogenic granuloma, denture hyperplasia)</td>
</tr>
<tr>
<td>B-2 microglobulin (component of histocompatibility antigen)</td>
<td>Cell surface of all epithelial layers</td>
<td>Partial loss</td>
<td>Loss</td>
<td>False-negative results&lt;br&gt;False-positive results</td>
</tr>
</tbody>
</table>

**Table 2:** Reaction pattern and limitations of the methods of intracellular antigenic constituents and products of squamous epithelium

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal mucosa</th>
<th>Dysplasia</th>
<th>Carcinoma</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin (large subunits)</td>
<td>Basal cells negative (thickening of keratin-negative basal cell compartment in hyperplasia)</td>
<td>Positive basal cells</td>
<td>Irregular distribution</td>
<td>Not diagnostic as such</td>
</tr>
<tr>
<td>Actin filaments</td>
<td>Basal cell layer positive</td>
<td>Increase in basal cells</td>
<td>Increase in invasive cell groups</td>
<td>Increased in wound healing and cellular locomotion</td>
</tr>
<tr>
<td>Virus coded products</td>
<td>-</td>
<td>Superficial layers positive</td>
<td>-</td>
<td>Questionable significance</td>
</tr>
<tr>
<td>CEA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Artifact due to cross reacting antigens</td>
</tr>
</tbody>
</table>

CEA: Carcinoembryonic antigen
3. The number of T-cells corresponds inversely with the presence or absence of metastasis in the regional lymph nodes.

**Immunoglobulins**

1. Plasma cells producing IgG and/or IgA accumulate in the connective tissue.
2. In certain cases, so do Russell bodies in the epithelial component; typical Langerhans cells are less numerous and exhibit a reduction of their complex dendritic network.

**Current predictive indicators**

1. Molecular (serologic/salivary and genomic):
   - p53 antibodies
   - Fragments of cytokeratin 19.
2. Morphologic (clinicopathological):
   - These are clinicopathologic features which help in predicting premalignant behavior.
3. Specific genes:
   - p53 gene
   - Proto-oncogenes.
4. Chromosomal losses and gains
5. Image-based ploidy analysis
6. Role of growth factors:
   - Fibroblast growth factors
   - Epidermal growth factor receptor (EGFR).
7. Proliferation markers:
   - Proliferating cell nuclear antigen
   - The nonhistone nuclear protein, Ki67 (MIB 1).

**Future Importance**

Immunoprevention has great potential for future cancer screening as driver mutations were detected in circulating cell-free DNA in patients with premalignant lesions (lung); clonal hematopoiesis shown to be a premalignant state; molecular selection in chemoprevention randomized controlled trial (RCT; oral).[9] Molecular biological markers, markers of epithelial differentiation, and genomic markers could be of great value in the diagnosis and prognostic evaluation of oral cancer.[10]

Expression of peroxiredoxin – 2, tyrosine kinase, and annexin A8 levels is elevated in the saliva of patients having oral cancer. So, using this testing in salivary proteins would be best and efficient method for detecting the preliminary stages of oral cancer.[10]

As the oral cavity malignancies are often not detected until an advanced stage, the survival rate for cancer of oral cavity has remained essentially unchanged through the past decades. Effective control of oral and oropharyngeal depends on prevention and early diagnosis. Continuing educational campaigns are needed on the local, provincial, and national level to educate the public about the early signs/symptoms associated with this disease. Oral screening by a dentist and/or physician supported with markers could transform oral cancer prevention and early detection.[11]

**Conclusion**

There are many promising aspects in the advanced methods presented. The grade of dysplasia of presumably premalignant lesions remains the most important indicator of a malignant potential. Any advanced method must be shown to predict the true biological potential more accurately than conventional histology.

**References**

7. Braakhuis BJ, Brakenhoff RH, Leemans CR. Second field

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**Table 3: Reaction patterns and limitations of the methods of antigenic constituents of the basement membrane zone and subepithelial tissue**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Normal mucosa</th>
<th>Dysplasia</th>
<th>Carcinoma</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin</td>
<td>Linear staining of basement membrane zone</td>
<td>-</td>
<td>Loss of polar deposition</td>
<td>Not diagnostic as such</td>
</tr>
<tr>
<td>Type IV collagen</td>
<td>Linear staining of basement membrane zone</td>
<td>-</td>
<td>Loss of polar deposition</td>
<td>Not diagnostic as such</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Network in the basement membrane zone</td>
<td>Breaks and fragmentation</td>
<td>Breaks and fragmentation</td>
<td>Not diagnostic as such</td>
</tr>
<tr>
<td>Procollagen</td>
<td>Staining of subepithelial tissue</td>
<td>Increase in amount</td>
<td>Increase</td>
<td>Not diagnostic as such</td>
</tr>
</tbody>
</table>