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Review Article

THE ROLE OF IMMUNOHISTOCHEMISTRY IN DIAGNOSIS OF VARIOUS ORAL LESIONS - A REVIEW

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ABSTRACT

The diagnosis of numerous lesions are essentially based on the microscopic study of cells and tissues. Hematoxylen and eosin staining is an established and age old, gold standard procedure for diagnosing all histopathological tissues. Despite this, H & E sections are undoubtedly insufficient at a diagnostic level when one engages in an etiologic, histogenetic or pathogenetic search, leading to invariable exploration for additional techniques. In the era when advanced immunologic and molecular techniques are used for diagnosis of disease and for its classification, pathologist relying entirely on the examination of tissue sections stained by histochemical methods is gradually being replaced. One such procedure is the immunohistochemistry which has revolutionized the field of surgical pathology during the past 50 years. A variety of markers are developed which are tissue/ organ specific like epithelial markers, lymphoid markers, vascular markers and are used successfully as diagnostic and prognostic markers of diseases. The main aim of this review is to enumerate and discuss the role of various markers used in the diagnosis of various oral lesions. The applied method of the search stratergies were books, printed articles, google, and pubmed database. The numbers of studies screened were fifty seven and included in the review were thirty nine, since the review was concerned only with oral potentially malignant disorders and oral cancer. This comprehensive review on role of immunohistochemistry in diagnosis of various oral lesions- A review, is attempted to highlight the importance of immunohistochemistry in diagnosis and prognosis of various oral lesions.

INTRODUCTION

Histochemistry is a science that combines the techniques of biochemistry and histology in the study of the chemical constitution of tissues and cells. Immunology is a science that deals with the immune system, cell-mediated and humoral aspects of immunity and immune responses. Immunohistochemistry (IHC) is the integration of the above mentioned disciplines. IHC is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody being identified either by direct labeling of the antibody, or by use of secondary labeling method.[1,2] Immunohistochemical staining of formalin-fixed and paraffin-embedded tissues is widely used in diagnostic surgical pathology.[3] Immunohistochemical methods in diagnostic surgical pathology has a long history.[4]

Immunohistochemical staining methods include use of fluorophore-labeled immunofluorescence) and enzyme-labeled (immunoperoxidase) antibodies to identify proteins and other molecules in cells. In diagnostic surgical pathology, immunoperoxidase methods (usually single antigen- antibody and less commonly double antibody- antigen combinations.[5, 6]

The aim of this review is to suggest that continuing reliance on immunohistochemistry in diagnosis of various lesions as biomarkers for predictive and prognostic studies and utility in the treatment protocol.

Principle of immunohistochemistry

It is the application of immunologic principles to techniques of histopathology. IHC utilizes antibodies and antibody based technology to detect and localize specific tissue antigens. An antigen has been defined as any substance which when introduced into the body, stimulates the production of the antibody with which it reacts specifically and in an observable manner.[1]

The basic principle of any IHC procedure is that an antibody will specifically bind with an antigen to produce an exclusive antibody-antigen complex. This bonding is used to visualize

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both normal and diseased states of tissues, infectious agents and other components that may not be demonstrated by histochemical or special stains.[2]

Working Classification of Antibodies Used In Immunohistochemistry [3,7, 8,9]

Epithelial Markers

Squamous epithelial

- Pancytokeratin.
- Carcino embryonic antigen.(CEA)
- Epithelial membrane antigen.(EMA)

Melanocytes

- S-100
- Melan –A
- Mart -1
- HMB - 45
- Tyrosinase

Odontogenic epithelium [10,11,12,13]

- Cytokeratin (ck) -5,13,14,19
  Basal layer and intermediate layer - ck 5, 14, 19
  Superficial cell layer - ck4,13
- Enamelin
- Ameloginin
- Laminin

Glandular epithelium [14]

- S-100
- Actin
- Calponin- myoepithelial cells
- CK 7
- Lysozyme.
- Estrogen receptors.
- Progesterone receptors.
- CK -14
- EMA- ductal cells
- P63- myoepithelial cells

Muscle Markers [15]

Skeletal muscle marker

- Myogenin (myf- 4, myf- 5)
- Myo D1

Smooth muscle marker

- Desmin
- Smooth muscle actin (SMA)
- Calponin
- Caldesmon

Vascular Markers [16]

- CD 31(Cluster of differentiation)
- CD 34
- CD105/ Endoglin
- VEGF
- Von willebrand factor (VIII)
- Ulex europaeus
- Podoplanin
- Podocalynin

Neural Markers[17]

- S-100
- Neuron specific enolase (NSE)
- CD57
- p75 NTR
- CD99
- CD56
- Neuro filament protein (NFP)
- Synaptophysin
- Chromogranin
- Claudin – 1
- Glut – 1.

Neuro Ectodermal Markers[17]

- CD99
- CD56/ NCAM - neural cell adhesion molecule
- NB84

Histiocytic / Dendritic Cell Markers [17]

- Lysozyme
- CD68
- CD21
- Clusterin,

Langerhans cell neoplasms express

- S100 protein
- CD1a.

Lymphoid Markers [18]

T cell markers

- CD 2
- CD3
- CD30
- CD99
- CD43
- CD45 RO
- TDT

B cell markers

- CD 20
- CD75 a
- CD26
- Pax5

Bone Markers [10,19]

- Receptor activator of nuclear factor-kappa B ligand (RANKL)
- CD99
- Osteocalcin
- Osteonectin
- Alkaline phosphatase.
- Osteoclast trap.
- Osteoprotegrin

Proliferative Markers Which Shows Up And Down Regulation [20]

Up regulation

- TGF – α (Transforming growth factor – alpha)
- PCNA (Proliferative cell nuclear antigen)
- Cyclin D1
- Ras

Down regulation

- p53
- TGF – β

Germ Cell Tumor Markers [21]

- Alpha feto protein(AFP)
- Human chorionic gonadotropin.(HCG) – In pregnancy.
- Germ cell tumor.
- Choriocarcinoma
- Islet cell tumor.
- CA125(Cancer Antigen)

Metastatic Epithelial Tumor Markers to Jaw [22]

- CK 7 and 20 – Low molecular weight cytokeratin
- Presence for metastatic lung and rectal adenocarcinoma.
- Villin - Actin binding protein.

It is difficult to recommend panels as the choice of antibodies will change according to the specific clinicopathological differential diagnosis. However, a few "basic" panels can be suggested, depending on the morphological category of the tumor:

Markers in spindle cell tumors [23]

- Smooth muscle actin
- Desmin
- S100
- Cytokeratins
- CD34
- EMA

Markers in Pleomorphic cell tumors[24]

- CK
- S100
- Actin
- Desmin
- CD30

Round cell tumors [25]

- CK
- Desmin and/or myf-4
- CD99
- S100

Markers salivary gland lesions (Intercalated duct lesions) [26]

- CK7
- CK14
- Lysozyme
- Calponin
- S-100
- Progesterone receptors.
- Estrogen receptors.

Other markers [26]

- CD99
- CD117 (transmembranous tyrosine kinase receptor)
- MDM- 2
- CDK-4
- FLI-1
- WT-1

Tumor markers can be broadly classified as [27]

1. Oncofetal antigens (e.g. alpha-fetoprotein (AFP), Carcinoembryonic antigen (CEA), Pancreatic oncofetal antigen, fetal sulfoglycoprotein.
2. Tumor associated antigens /Cancer Antigens e.g.CA125, CA19-9, CA15-3, CA72-4 CA50 etc.
3. Hormones e.g. Beta human chorionic gonadotropin, calcitonin, placental lactogen etc.
4. Hormone receptors (e.g. estrogen and progesterone receptors)
5. Enzymes and Isoenzymes (e.g. prostate specific antigen (PSA), prostatic acid phosphatase (PAP), neuron specific enolase (NSE), glycosyl transferases, placental alkaline phosphatase (PALP), terminal deoxy nucleotidyl transferase (TDT), lysozyme, alpha amylase
6. Serum and tissue proteins (beta-2 microglobulin, monoclonal immunoglobulin/para proteins, glial fibrillary acidic protein (GFAP), protein S-100, ferritin, fibrinogen degradation products)
7. Other biomolecules e.g. polyamines

Intermediate Filaments

The intermediate filaments comprise the major component of the cytoskeleton and consist of five major subgroups [vimentin, cytokeratins, desmin, neurofilaments, glial fibrillary acidic protein (GFAP)] and a small number of minor subgroups (e.g. nestin, peripherin). Ultastructurally, the intermediate filaments appear as wavy unbranched filaments that often occupy a perinuclear location in the cell.[3]

Vimentin

Vimentin, a 57-kDa intermediate filament protein, has been expressed by all mesenchymal cells. Vimentin is universally expressed in all most all the cells during early embryogenesis and is progressively been replaced in many cells by type-specific intermediate filaments. Few mesenchymal tissues express vimentin with the type-specific intermediate filaments. It is also commonly expressed by sarcomatoid carcinomas at any site, an unfortunate fact that greatly limits its utility in the immunohistochemical distinction of carcinomas from sarcomas.[28]

Cytokeratins

Cytokeratin are the most complex members of the intermediate filament protein family. They are a group of more than 20 proteins. The cytokeratins may be grouped by their molecular weights (40-67 kDa) into **acidic and basic** subfamilies. The cytokeratins are mostly grouped as **low-molecular-weight cytokeratins** (generally cytokeratins 8, 18, and 19) and **highmolecular weight cytokeratins** (generally cytokeratins 1, 5, 10, and 14). Cytokeratins are highly sensitive markers for

identifying carcinomas and are generally employed as markers distinguishing epithelial from non epithelial tumors (lymphomas, sarcomas, melanomas). Cytokeratins is expressed in only a portion of cytoplasm, often yielding a "PERINUCLEAR" OR "DOT LIKE PATTERN" typically in merkel cell tumors.[29]

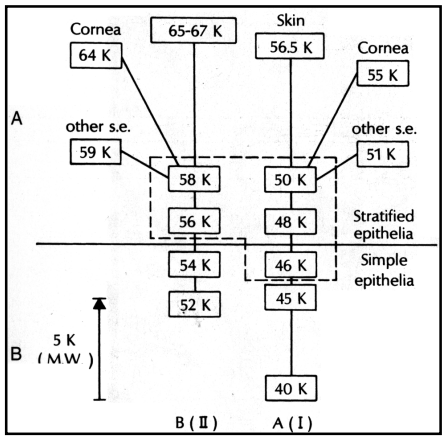

Figure 1 Showing subcategorization of acidic (A) and basic (B) cytokeratin subgroups within various tissues.[3]

Epithelial membrane antigen

Epithelial membrane antigen (EMA) belongs to a heterogeneous family of highly glycosylated transmembrane proteins known as human milk fat globule (HMFG) membrane proteins. This family of antigens is high molecular weight molecules found in secretory epithelial cells, to a lesser degree, in non-secretory epithelium (e.g., squamous epithelium) and rarely in non-epithelial cells.[30] Among normal mesenchymal cells, EMA expression is limited to perineurial cells, meningeal cells.Tumors which can be detected are Perineuromas, malignant peripheral nerve sheath tumors, synovial sarcoma, meningiomas, angiomatoid fibrous histiocytoma, dentigerous cyst, KCOT, osteosarcoma, Neurofibromas, pleomorphic adenoma, salivary duct carcinoma, polymorphous low grade carcinoma, basal cell carcinoma, adeno carcinoma, clear cell carcinoma.[31]

Desmin

Desmin is the intermediate filament protein associated with both smooth and skeletal muscle differentiation; it is rarely expressed by myofibroblasts and their corresponding tumors. Many molecules have been reported to associate with desmin, such as nebulin, the actin and tubulin binding protein plectin, the molecular motor dynein, the gene regulatory protein MyoD, DNA, the chaperone αB-crystallin, and proteases such as calpain and caspase.[32] In skeletal muscle desmin is localized to the Z-zone between the myofibrils, where it presumably serves as binding material for the contractile apparatus.[33] Desmin may also be expressed by non muscle cells, including the fibroblastic reticulum cell of the lymph node, submesothelial fibroblast, and endometrial stromal cells. Tumors which can be detected are Benign & malignant smooth & skeletal muscle tumors, especially Rhabdomyosarcoma.[32]

Actin

Actin, also is expressed by both smooth and skeletal muscles. Actin microfilaments transmit traction and contraction forces generated within a cell to the extracellular matrix during embryonic development, wound healing and cell motility, and to maintain tissue structure and tone. The tumors which can be detected are Leiomyoma & sarcoma, rhabdomyoma & sarcoma, mucoepidermoid sarcoma, salivary duct carcinoma, adenoid cystic carcinoma.[34]

Markers of Nerve Sheath Differentiation S-100 Protein

The S-100 protein is a 20-kDa acidic calcium-binding protein, so named for its **solubility in 100% ammonium sulfate.** The protein is composed of two subunits, α and β. S-100 protein can be demonstrated in a large number of normal tissues, including some neurons and glia, schwann cells, melanocytes, Langerhans cells, interdigitating reticulum cells of lymph nodes, chondrocytes, myoepithelial cells and ducts of sweat glands, salivary glands, and the breast, serous glands of the lung, fetal neuroblasts and sustentacular cells of the adrenal medulla and paraganglia.[3] The tumors which can be detected are Benign & malignant nerve sheath tumour, melanoma, schwannoma, chondrosarcomas, langerhans cell tumour, Neurofibromas, some muscle tumors, pleomorphic adenoma, basal cell adenoma, mucoepidermoid carcinoma, adenoid cystic carcinoma, adeno carcinoma.[35]

Markers of melanocytic differentiation HMB-45

Monoclonal antibody HMB-45 is a monoclonal antibody and is used to detect an epitope specific for melanocyte, malignant melanoma and melanoma metastasis. The reactive agent is present in the cutaneous melanocytes, melanoma cells and pigments in retina.[36]

Vascular Markers

CD34 (Human Hematopoietic Progenitor Cell Antigen)

The function of CD34, a 110-kD a transmembrane glycoprotein, is unknown. It is expressed on hematopoietic stem cells, endothelium, the interstitial cells of Cajal, and a group. 'of interesting dendritic cells present in the dermis, around blood vessels, and in the nerve sheath.[3] CD34 is expressed in more than 90% of vascular tumors and is the most sensitive marker of Kaposi's sarcomaCD34 positive cells were also found in prostate, urinary bladder, fallopian tubes, thyroid gland, pancreas, colon, uterine cervix and testis.[37]

CD31 (Platelet Endothelial Cell Adhesion Molecule-1)

CD31 (PECAM-1; platelet/endothelial cell adhesion molecule-1) is a single chain type-1 transmembrane protein that plays a role in adhesive interactions between adjacent endothelial cells as well as between leukocytes and endothelial cells.[38] It is expressed in more than 90% of angiosarcomas, hemangioendothelioma hemangiomas, and Kaposi's sarcoma.[3]

VEGF

VEGF is a type of glycoprotein which has both angiogenic, mitogenic as well as vascular permeability factor thus enhancing activity in endothelial cells. It serves as an important marker in premalignant lesions (leukoplakia, lichen planus), malignant (oral squamous cell carcinoma), salivary gland tumors, odontogenic cysts and tumors, kaposis's sarcoma and lymphangioma.[39,40,41]

CONCLUSION

Various markers are used in one or more lesions and are not organ specific. Virtually no IHC marker has been accepted and shown to be specific or sensitive enough to use in the detection of specific tumors in the common population. The application of immunologic research methods to histopathology has resulted marked improvement in the microscopic diagnosis of neoplasms. Although histologic analysis of hematoxylin and eosin stained tissue sections remains at the core of the practice of head and neck surgical pathology, immunohistochemistry has become powerful tool in the armamentarium of the pathologist. In any event, immunohistochemical studies must be interpreted only in the context of the impression made after review of H & E sections. More advanced techniques are used along with IHC like polymerase chain reaction (PCR), northern blot technique, southern blot technique, micro array analysis etc for the ease of diagnosis and treatment plan. Thus all these technology will help to remove the misinterpretation in diagnosis and will improvise in better treatment and prognosis of the disease.

References

1. Bancroft D.J theory and practice of histological techniques 6th edition
2. Applied Immunohistochemistry for the surgical pathologist, Anthony S-Y Leong
3. Enzinger & Weiss's Soft tissue tumors, 5th edition.
4. Idikio HA. Immunohistochemistry in diagnostic surgical pathology: contributions of protein life-cycle, use of evidence-based methods and data normalization on interpretation of immunohistochemical stains. *Int J Clin Exp Patho*l 2010; 3(2):169-176
5. Robinson G, Dawson I. Immunochemical studies of the endocrine cells of the gastrointestinal tract II An immunoperoxidase technique for the localization of secretin-containing cells in human duodenum. *J Clin Patho* 1975; 28: 631-35.
6. Hanahan D, Weinberg R. The Hallmarks of Cancer. Cell 2000; 100:57-70.
7. Richard C. K. Jordan, *et al*. Advanced diagnostic methods in oral and maxillofacial pathology. Part II: Immunohistochemical and immunofluorescent methods. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:56-74
8. Saint N. 2005.Aubain Somerhausen; Institut Jules Bordet /Hôpital: Erasme: nicolas. desaintaubain@synet.be
9. Shailja Chatterjee. Cytokeratins in health and disease. *Oral & Maxillofacial Pathology Journal* 2012; 3(1):198-202
10. Orban's oral histology and embryology. eleveenth edition. Elsevier publication.
11. RIECHART.PA, Philipsen HP, Odontogenic tumors and allied lesions Quintessence Publishing Co Ltd.P-53-5
12. Tencate's Oral Histology, development, structure and function.8th Edition
13. WHO classification of Head and neck tumors. Barnes L, Eveson JW, Reichart .P, Sidransky. D. IARC press, Lyon 2005.P-165-8
14. Ellis GL, Auclair PL, Douglas R. Gnepp. Surgical Pathology of the Salivary Glands Saunders, 1991
15. Fletcher CDM.Diagnostic histopathology of tumors. Second edition.vol.1,2 Tarazendah medical publication
16. Gupta B, Chandra S, Raj V, Gupta V.Immunohistochemical Expression of Vascular Endothelial Growth factor in Orofacial Lesions: a Review. *J Oral Biol Craniofac Research* 2016
17. Miller RT. Immunohistocemical Approach to "undifferent tumors". American Academy of Oral and Maxillofacial Pathology Annual Meeting San Juan, Puerto Rico Saturday, April 30, 2011
18. Rosai and Ackerman's surgical pathology ninth edition. Tarazendah medical publication. p- 1884-7
19. Wheater G, Elshahaly M, Tuck SP, Datta HK, van Laar JM, The clinical utility of bone marker measurements in osteoporosis. *Journal of Translational Medicine* 2013;11:201
20. Robbins and Cotran. Pathologic basis of disease. Seventh edition. Elsevier Saundres, Philadelphia
21. Kawai M, Kano T, Kikkawa F, Morikawa Y, Oguchi H, Nakashima N, Ishizuka T, Kuzuya K, Ohta M, Arii Y. Seven tumor markers in benign and malignant germ cell tumors of the ovary Gynecol Oncol. 1992 Jun; 45(3):248-53.
22. Hirshberg A, Berger R, Allon I, Kaplan I. Metastatic Tumors to the Jaws and Mouth. Head Neck Pathol. 2014; 8(4): 463–74.
23. Hall JH, Yohe SL. Application of Immunohistochemistry to Soft Tissue Neoplasms. Arch Pathol Lab Med. 2008;132:476–89
24. Hornick JL. Novel uses of immunohistochemistry in the diagnosis and classification of soft tissue tumors Modern Pathology.2014;**27**
25. Rajwanshi A, Srinivas R, Upasana G. Malignant small round cell tumors. *J Cytol*. 2009; 26(1): 1–10.
26. Cohn SL Diagnosis and Classification of the Small Round-Cell Tumors of Childhood. *Am J Pathol*. 1999; 155(1): 11–15
27. Malati.T. Tumour markers: An overview. Indian Journal of Clinical Biochemistry. 2007; 22(2):17-31)
28. Schnitzer J, Franke WW, SCHACHNER M. Immunocytochemical Demonstration of Vimentin in Astrocytes and Ependymal Cells of Developing and Adult Mouse Nervous System. *The journal of cell biology*.1981;90:435-447
29. Chatterjee S. Cytokeratins in health and disease. OMPJ.2012;3(1):976-1225
30. Al-Abbadi MA, Almasri NM, Al-Quran S, Wilkinson EJ. Cytokeratin and Epithelial Membrane Antigen Expression in Angiosarcomas an Immunohistochemical Study of 33 Cases. Arch Pathol Lab Med. 2007;131:288–92
31. Pinkus GS, Kurtin PJ. Epithelial membrane antigen--a diagnostic discriminant in surgical pathology: immunohistochemical profile in epithelial, mesenchymal, and hematopoietic neoplasms using paraffin sections and monoclonal antibodies. Hum Pathol. 1985 Sep; 16(9):929-40.

32. Costa M.L, Escaleira R, Cataldo.A, Oliveira.F, Mermelstein.CS. Desmin: molecular interactions and putative functions of the muscle intermediate filament protein. *Brazilian Journal of Medical and Biological Research*.2004; 3 7:1819-30
33. Stromer MH & Bendayan M (1988). Arrangement of desmin intermediate filaments in smooth muscle cells as shown by high-resolution immunocytochemistry. Cell Motility and the Cytoskeleton, 11: 117-125
34. Wakatsuki T, Schwab B, Thompson NC, Elson. EL. Effects of cytochalasin D and latrunculin B on mechanical properties of cells. Journal of cell science. Journal of Cell Science.2000; 114 (5):1025-36
35. Chen H, Xu C, Jin Q, Liu Z. Review Article S100 protein family in human cancer. *Am J Cancer Res* 2014;4(2):89-115
36. Schwechheimer.K, Zhou.L. Aspecific marker for melanoma metastasis in the central nervous system?1995; 426:351-53
37. Cimpean AM, Raica M, Narița.D. Diagnostic significance of the immunoexpression of CD34 and smooth muscle cell actin in benign and malignant tumors of the breast. *Romanian Journal of Morphology and Embryology*.2005;46(2):123–29
38. Pisacane A.M, Picciotto F. Risio M. CD31 and CD34 expression as immunohistochemical markers of endothelial transdifferentiation in human cutaneous melanoma. Cellular Oncology.2007;29:59–66
39. Gupta B, Chandra S, Raj V, Gupta V.Immunohistochemical Expression of Vascular Endothelial Growth factor in Orofacial Lesions: a Review. *J Oral Biol Craniofac Research* 2016.(In press)
40. Ferrara N. Vascular Endothelial Growth Factor: Basic Science and Clinical Progress. Endocrine Reviews 2004; 25(4):581-611.
41. Ferrara N, Davis ST. The biology of vascular endothelial growth factor. Endocr Rev 1997; 18:4-25.

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