Applications Of Immunohistochemistry-A Review

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ABSTRACT:

Immunohistochemistry can be an important method in diagnosis, investigation, and determining the behavior and pathogenesis of oral tumors. Immunohistochemistry protocols were formed using antibodies tagged with chromogens to identify particular markers. In these protocols, antigen-antibody reactions occur using nonfluorescent chromogens and then are analyzed in an optical microscope. Subspecialties of pathology like in that of oncologic pathology, neuropathology, and hematopathology, gradual increase in need of IHC can be observed as time goes by. As specific diagnostic markers can be present extensively in cells of a particular neoplasm and not in other tumors thus these markers can be used to find out the cellular lineage and histogenic origin of different neoplasms. This paper focuses mainly on the Applications of Immunohistochemistry in Oral Pathology.

KEYWORDS: oral pathology, immunohistochemistry, tumor markers, keratins

INTRODUCTION:

The first IHC was reported in the date back to 1941 though principle of IHC has been known since 1930. [1] Coons and his colleagues utilized Fluorescein isothiocyanate (FITC)-labeled antibodies with a fluorescent dye to locate pneumococcal antigens within infected tissues. Gradually with the expansion and growth of IHC technique, enzyme labels have been introduced, such as peroxidase[2,3] and alkaline phosphatase[4] .Colloidal gold[5] label has also been discovered and used to detect immunohistochemical reactions both at light and electron microscopy levels. The contribution of immunologic research methods to histopathology has led to remarkable improvement in the microscopic diagnosis of neoplasms. Still histologic analysis of hematoxylin and eosin stained tissue sections remains as the gold standard for head and neck surgical pathology, in spite of that immunohistochemistry has becoming a powerful tool in the armamentarium of the pathologist.[6] It affords a significant advantage in the diagnosis of difficult and equivocal tumors.Application of immunohistochemistry in distinguishing undifferentiated oral neoplasms of different origins was achieved through the detection of tumor antigens using known antibodies[7] Thus, immunohistochemistry is important in diagnosis, investigation, and determining the behavior and pathogenesis of oral tumors.

APPLICATIONS:

IHC has become an important technique and is widely utilized in many medical research laboratories and also in clinical diagnostics[8].IHC involvement with specific antigen–antibody reactions apparently brings about advantage over traditionally used special enzyme for staining techniques that can identify only a few limited number of proteins, enzymes, and tissue structures.Its contribution in various oral lesions are as follows:-

PROGNOSTIC MARKERS OF IHC:

Through identification of enzymes, tumor-specific antigens, oncogenes, tumor suppressor genes, and tumor cell proliferation markers prognosis of tumors can be successfully detected. Analysis of tumors with the help of these methods poses a significant improvement over this conventional techniques of clinical staging and histologic grading. IHC is utilized for disease diagnosis, development of drug, and biological research. By means of using specific tumor markers, physicians use IHC in order to diagnose whether the particular neoplasm is benign or malignant, and if positive then help to determine the stage and grade of a tumor, as well as identify the cell type and origin of a metastasis to find the site of the primary tumor. IHC can also be used in drug development to assess drug efficacy by either detecting the activity or the up or down-regulation of disease targets[9].

TUMORS OF UNCERTAIN HISTOGENESIS:

The particular approach of IHC methods in diagnosis of different tumors of uncertain origin and primary as well as metastatic tumors from unknown primary neoplasm is considered to bring about a revolutionary change in methods of diagnosis and treatment planning. In order to resolve such diagnostic problem cases a panel of antibodies is chosen. The selection of antibodies generally made on the basis of clinical history, morphological features, and results of other relevant investigations. Immunohistochemical stains in case of intermediate filaments are expressed by tumor cells (keratin, desmin, vimentin, neurofilaments, and glial fibrillary acidic proteins) [9]

INFECTIONS :

To confirm infectious agent in tissues by using specific antibodies against microbial DNA or RNA IHC methods can be handy, e.g. as seen in Cytomegalo virus, Hepatitis B virus, Hepatitis C virus, etc. since 1940s with the help of tagging fluorescein dye (visible under ultraviolet light) to antibodies which is then directed against the pneumococci to help in the identification of this particular organism with specific anti-serum[1] .This method, often abbreviated IFA for "immunofluorescence assay", has been widely used for the detection of specific pathogens, can be viral as well as bacterial and protozoal, in unfixed tissues or obtained in fresh from both human and veterinary medicine. Another important advantage of IHC is that it can identify organisms in cytological preparations such as fluids, sputum samples, and material obtained from fine needle aspiration procedures. This can be made utilized in certain situations like while detecting pneumocystis from the sputum of an immunocompromised patient needing a rapid and precise confirmation of infection in order to proceed with immediate and appropriate therapy.

ORAL SQUAMOUS CELL CARCINOMA :

Squamous cell carcinoma is by far the most common form of oral cancer. Immunohistochemical markers of interest in squamous cell carcinoma are keratins (the cytoskeletal proteins of epithelial cells), other squamous cell carcinoma antigens, and markers applicable in the evaluation of the biologic potential of tumors, such as proliferation antigen.[10]

Keratin:

The intermediate filament proteins of cytoskeleton typical in all epithelial cells are Keratins. More than 20 different keratins are known to be located in epithelial cells[10,11] These are the polypeptides which are numbered from 1 to 20 comprising the type II (basic) and the type I (acidic) keratins. This family of intermediate filaments is crucial in diagnostic immunohistochemistry for the identification of specific carcinoma subtypes [12] .The normal oral squamous epithelium contains predominantly high molecular weight keratins. Keratins 5, 14, and 19 are present in the basal cell layer; and keratin 4 and 13 in the upper layer, together with other keratins[6]. Oral squamous cell carcinomas typically possess high molecular weight keratins which is basically CK5 and CK14 and keratin 19 in most cells in comparison to the basal distribution in normal squamous epithelia. The appearance of low molecular weight keratins 8 and 18 can also be seen often in oral squamous cell carcinoma which sometimes

appear that too focally in normal oral epithelia. Keratin 19 expression is considered to be non specific for detecting premalignancies since its level is seen raised both in oral premalignancy and also in hyperplastic lesions [10,11] .Poorly differentiated carcinoma that may resemble sarcoma can be identified by its content of keratins of high and low molecular weights. Reactivity with antibodies to high molecular weight keratins suggests squamous cell differentiation of a carcinoma, whereas low molecular weight keratins are limited only to adenocarcinomas.[3] To differentiate adenocarcinomas from the adenoid squamous cell carcinomas keratin 20 can be used since it is negative in squamous cell carcinoma[12] .Along with tumor typing various immunohistochemical markers can be used to assess the proliferation potential of the tumors.

Markers of cellular proliferation and biologic potential

Squamous cell carcinomas have always been the main objects of immunohistochemical studies in comparison to all other oral neoplasms thus evaluating the specific markers of cell proliferation and their possible prognostic correlations are mandatory. Proliferation of a cell mainly depends on the cell cycle. Many nuclear proteins are differently expressed in various stages of the cell cycle which includes DNA duplication while cellular replication. Originally it has been reported that Ki-67 antigen, a nuclear protein, was isolated from Reed–Sternberg's cell line which has shown to be expressed in all cells except for those in G0 phase (resting, noncycling cells) [12]. Ki-67 staining gives minimal background staining and more contrast hence becomes easier to interpret [12] .On contrary Proliferating cell nuclear antigen (PCNA), a member of the cyclin family, is an auxiliary component of DNA-polymerase-δ and it appears in all cycling (proliferating) cells therefore, it detects a higher number of cycling cells than Ki-67. Furthermore, it has long half-life (20 h) that results in staining of cells which have recently departed the cell cycle and influenced by primary antibody dilution and fixation conditions. Hence, Ki-67 is a reliable tool for measuring proliferative activity in human tissue [10] .The immunoreactivity of these proliferation markers appears limited to the proliferating basal cells both in the normal and hyperplastic mucosa, but it gradually increases and also appears in suprabasal layers in the dysplastic mucosa. One study suggested that the PCNA score decreased after chemotherapy, suggesting that, this marker might be useful in monitoring the response to therapy.[5]

TURES OF ORAL SALIVARY GLAND TUMORS

The salivary glands host an extraordinarily diverse array of neoplasms. Most are proliferations of ductal and myoepithelial cells in various combinations; acinar cells participate infrequently. Interpretation of histologic material is further complicated by the fact that a variety of secondary alterations is common to several different entities. They include clear cell change, cystic change, oncocytic cytologic features, sebaceous differentiation, and prominent lymphoid stroma. To some extent, immunohistochemistry can solve the confusion regarding the typing of salivary gland tumors and aid in correct diagnosis and prognosis in few cases [11]

Pleomorphic adenomawhich is considered to be the most common benign salivary gland tumor consist of components like ductal and myoepithelial cells which show immune-reactivity to various markers. Staining for glial fibrillary acidic protein (GFAP) has been confined to the myoepithelial calls that appear ultra structurally to be the most undifferentiated, in a pattern similar to neoplastic cartilage. This marker has proved diagnostically useful in both histopathologic and in fine-needle aspiration cytologic studies [12] .The majority of pleomorphic adenomas are positive, whereas normal salivary gland tissue, chronic sialadenitis, basal cell adenomas,adenoid cystic carcinomas, and low-grade mucoepidermoid carcinomas give negative results. This marker has utility in differentiating the pleomorphic adenomas from the polymorphous low-grade adenocarcinomas [13] .As it can be confused with the pleomorphic adenoma, polymorphous low-grade adenocarcinomas may simulate histologically with adenoid cystic carcinoma. Adenoid cystic carcinoma is different immunohistochemically by showing extensive immunoreactivity for S-100 protein, muscle actins, and epithelial membrane antigen; these antigens are seen in a more limited pattern in adenoid cystic carcinoma [13] .Furthermore, Ki-67 immunostaining can be helpful in their differentiation **[14]** .Thus, the role of immunohistochemistry in salivary gland tumors is limited but important **[15]**

ORAL MELANOMA:

Malignant melanoma can present as a primary tumor in oral mucosa or as a metastasis in adjacent soft tissues and jaw bones. The histologic diagnosis may be difficult, especially in cases in which the biopsy shows no junctional connection with the surface epithelium and the tumor is amelanotic. Histologic patterns of melanoma also varies and include epithelioid, spindle cell sarcomatous, or round cell appearances that must be distinguished from carcinoma, sarcoma, and lymphoma, respectively. Malignant melanoma has a typical antigenic profile, and this diagnosis usually can be confirmed easily by immunohistochemistry [17] .S-100 protein is present in almost 95% cases. They are also positive for neuron-specific enolase (NSE) and vimentin and usually are negative for keratin. Melanoma-specific antigen identified with monoclonal antibody homatropine methylbromide-45 (HMB-45) is present in about 70-80% of melanomas, especially the so-called desmoplastic (neurotropic) melanomas that may simulate soft tissue sarcomas[12] Furthermore, 75% melanomas are positive for MELAN-A or anti-tyrosinase. Among these S-100 protein is highly sensitive but not specific, whereas HMB-45 is highly specific and moderately sensitive[18] .Clear cell sarcomas, or melanoma of the soft parts, are unique tumors producing melanin and are intimately associated with tendons or aponeuroses. They express S-100 and often HMB-45, NSE, and Leu-7. The absence of mucin and the presence of melanin distinguish them from synovial sarcomas [19] [16]

BENIGN MESENCHYMAL TUMORS OF ORAL CAVITY:

Certain tumors among the mesenchymal tumors require immunohistochemistry for their identification and differentiation from other tumors. Examples are namely the granular cell tumor, granular cell epulis, melanotic neuroectodermal tumor of infancy and schwannoma. Granular cell tumor which is formerly known as granular cell myoblastoma occurs within the oral cavity, commonly in the tongue of the adult patient. This particular tumor is considered to be related to Schwann cell neoplasms and shows positivity for S-100 protein and vimentin that is similar to schwannoma, but marks negative for the muscle cell markers desmin and muscle actins, consistent with its Schwann cell, and nonmuscular nature [10] Granular cells are also positive for NSE, laminin, and myelin basic proteins. Staining is negative for neurofilament proteins and GFAP [18] .Granular cell tumor of gingiva of newborns (granular cell epulis) which usually presents in the anterior alveolar ridge of newborns differs from granular cell tumor of adults and is believed to be of fibroblastic origin [13]. This tumor is positive for vimentin but in contrast to granular cell tumor of adults is negative for S-100 protein [6,13]. Melanotic neuroectodermal tumor of infancy also called as melanotic prognoma is a rare, usually benign tumor that occurs in the jawbones, most commonly in infants. This tumor characterized by small neural-like cells and large, pigmented epithelial-like cells and shows a complex immunohistochemical profile reflecting its divergent differentiation properties. The large cell or the cuboidal cell component marks positive for keratins and melanoma-specific antigen (HMB-45), but show usually negative for S-100. Some small cell component is also positive for vimentin, epithelial membrane antigen, GFAP, NSE, and synaptophysin[6,13]

ORAL SARCOMAS

Both soft tissue sarcomas of different types and bone sarcomas arising in jaw bones may present in the oral cavity. Among the various sarcomas many sarcomas occur in the oral cavity, but before oral sarcoma is diagnosed, it is important to exclude the possibility of sarcomatoid spindle cell carcinoma and malignant melanoma. Most of the sarcomas possess vimentin which is the intermediate filament protein typical of mesenchymal cells and negative for keratins. Moreover as described previously, melanoma is positive for S-100 protein and melanoma-specific antigen (HMB-45) [20]

OSTEOSARCOMA

Considered to be one of the greatest challenges in the bone and soft tissue tumor pathology is the reliable recognition of osseous matrix production in malignant lesions. Because the presence of true osteoid equates with a diagnosis of osteosarcoma, this is an important issue. Since the late 1990s, number of putatively osteoblast-specific markers have been developed including bone morphogenetic protein, type I collagen, COL-I-C peptide, decorin, osteocalcin, osteonectin, osteopontin, proteoglycans I and II, bone sialoprotein, and bone glycoprotein

75. Among these, only two-osteonectin and osteocalcin (OCN) have been associated with sufficiently good performance in paraffin section to merit their inclusion in diagnostic immunohistologic studies. OCN is one of the most prevalent noncollagenous intra-osseous proteins and is predominantly localized to osteoblasts. OCN generally has a reasonable level of sensitivity for osteoblastic differentiation (approximately 70%) and is, for practical purposes, virtually completely specific for bone-forming cells and tumors. However, sometimes fibroblasts can also cross-react with the polyclonal anti-OCN reagents therefore; monoclonal antibodies with selective peptide recognition are preferred for diagnostic work. Thus, it can be used with reasonable success as a single marker to detect such neoplasms[7]

VASCULAR MALIGNANCIES

Malignancies of vascular tissue are the next one although rare but can pose definitive diagnostic difficulties due to a wide spectrum of morphologic appearances. Angiosarcoma is the rare malignant tumors recapitulating the features of endothelial cells. Among the antigens most useful in identification of angiosarcomas is CD31, the platelet-endothelium cell adhesion molecule-1. This antigen is present in endothelial cells and platelets but is practically absent in carcinomas and is detectable in about 90% of angiosarcomas and also in Kaposi's sarcoma [21] CD34 (hematopoietic progenitor cell antigen) is present in endothelial cells and in some fibroblasts. CD34 is present in about 80-90% of angiosarcomas and Kaposi's sarcoma, but is also present in other sarcomas, especially in epithelioid sarcoma, dermatofibrosarcoma protuberans, and leiomyosarcoma[12]. A new marker FLI1, a nuclear transcription factor appears to be expressed in almost 100% of different vascular tumors, including Kaposi's sarcoma[22]

HEMATOLOGICAL MALIGNANCIES

Lymphomas

These are often diagnosed as undifferentiated malignant neoplasms. Immunohistochemistry plays a decisive role in oral lymphomas. The main problem in the instance of small cell lesions is to differentiate them from reactive lymphoid proliferations, which is done by documenting the clonal nature of the proliferations. Large cell lymphomas, in turn, must be differentiated from other undifferentiated-appearing malignant neoplasms, such as carcinomas and melanomas using antibodies to LCA (LCA, CD45), keratin and S-100 protein [10] LCA is an excellent screening marker for lymphoid cells, including the non-Hodgkin's lymphomas. LCA positive cells can be further delineated by more specific lymphoid markers, including those for T-lymphocytes (UCHL-1, CD3, L60, and MT1), B-lymphocytes (L26, LN1, LN2, LMB1, and MB2**)[19]** Most of the small lymphomas are or B-cell origin and show monotypic immunoglobulin light chains (κ or λ), which can be identified consistently on acetone fixed frozen sections. Immunohistochemistry is also helpful in identification of Hodgkin's disease. Markers for Reed–Sternberg cells include CD15, CD30, and BLA36. In lymphocyte predominant Hodgkin's disease and the Reed–Sternberg cells differ in phenotype and are CD15, CD30 negative but CD45, CD20 and BLA36 positive [23]

Peripheral Nerve Tumors

In contrast to neurofibromas, which contain a mixture of cells, neurilemmomas consist predominantly of Schwann cells (schwannoma) and, therefore, express S-100 protein, variably Leu-7, and occasionally GFAP. Leiomyosarcomas can show some histological resemblance, but they generally do not express S-100 protein. Neurofilament protein helps to distinguish between neurilemmomas and neurofibromas.

CONCLUSION:

Thus this Review provides a brief overview of the role of immunohistochemistry in various oral lesions, benign as well as malignant. However as days going by many new antibodies are being commercialized. It is believed that in the near future, the pathologist will play a central role in diagnosis and immunohistochemistry as the routine armamentarium in diagnostic tests. Thus, these changes likely will improve the understanding of diseases that affect the head and neck and the ability of the pathologist to render a correct diagnosis.

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