IMMUNOHISTOCHEMICAL EXPRESSION OF CD44 IN PLEOMORPHIC ADENOMA AND MUCOEPIDERMOID CARCINOMA

Thesis Submitted to the Faculty of Dentistry, Ain-Shams University for Partial Fullfilment of the Requirements of the Master's Degree in Oral Pathology

BY

Hala Ahmed EL-Kammar

B.D.S.

2002

Faculty of Dentistry

Ain-shams University

2009

Supervisors

Prof. Dr. Mohamed Salah El-Din Ahmed Ayoub

Professor of Oral Pathology and Vice-Dean for post graduate studies of Faculty of Dentistry, Ain Shams University

Dr. Marwa Mokbel El Shafei

Lecturer of Oral Pathology, Faculty of Dentistry, Misr International University

Dedications

I am grateful to God for enabling me to finish this work and for blessing me with a loving and supporting family.

بسم الله الرحمن الرحيم

AIM OF THE STUDY

The aim of the present study was to:

1) Detect and correlate the expression of CD44 in Pleomorphic adenoma
2) Detect and correlate the expression of CD44 in different grades of Mucoepidermoid carcinoma.
3) Compare CD44 expression in Pleomorphic adenoma and Mucoepidermoid carcinoma.

INTRODUCTION AND REVIEW OF LITERATURE

An organ is a mass of diverse cells possessing various morphological and functional identities. The diversity of cells ensures that organs develop a highly elaborate multicellular system to adapt complex physiological demands. An important premise involved in this system is the harmonization of each cell activity to achieve organ function adequately and efficiently. Organ function requires coordinated multicellular activities, which may require proper control of cell signaling dynamics at the supracellular level[(1)].

Salivary glands

The salivary gland is an exocrine gland capable of secreting fluid and macromolecules under the autonomic nerve regulation. Salivary gland function is accomplished primarily by the compartmentalized epithelial domains, acini and ducts, the former involved in the production of primary saliva and the latter involved in its modification[(1)].

Saliva is transported from the central structure of the salivary unit, the acini, in a complex ductal system to the oral cavity. The system is bilayered with an internal luminal layer and an external reserve layer. The internal layer forms the acini and the ductal epithelium, while the external layer forms the myoepithelium and the reserve cells[(1)].

There are two theories regarding the pathogenesis of salivary gland tumors. The reserve cell theory postulates that neoplasms arise

from the reserve cells, which act as the stem cells for salivary glands. While, the Multicellular theory states that, striated ducts give rise to oncocytic tumors, acinar cells to acinic cell carcinomas, excretory ducts to squamous cell and mucoepidermoid carcinomas, and the intercalated duct and myoepithelial cells to pleomorphic adenomas[(2)].

Salivary gland tumors are uncommon. The tissue organization of the salivary gland is complex, and a large number of salivary gland tumor entities with a broad morphologic spectrum are listed, creating tumor classification schema for the salivary glands that are difficult to understand[(3)].

The more recent WHO classification Divides the salivary gland tumors into; Adenomas, Carcinomas, Non-epithelial tumors, Malignant Lymphomas, Secondary tumors, Unclassified tumors and tumor like lesions[(4)].

Epidemiological data show that, Out of all salivary gland tumors, benign tumors are more common than malignant ones. The majority of cases occur in the parotid gland, followed by the minor salivary glands then the submandibular gland. Among the minor salivary gland tumors, the palate is the most frequent location. The tumors affected more commonly adult patients with peak incidence between 40 and 50 years of age and with a slight predominance in females. Pleomorphic adenoma was the most frequent tumor, followed by mucoepidermoid carcinoma, Warthin's tumor and adenoid cystic carcinoma[(5)].

The etiology of salivary gland carcinoma (SGC) is not well known, although correlations of varying strengths between SGC and possible risk factors have been found. One of the most well-established risk factors of SGC is exposure to radiation. High-dose radiation has been conclusively linked to SGC in studies of atomic bomb survivors, with a strong dose-response seen for mucoepidermoid carcinomas. Therapeutic radiation has also been linked to an increased risk of SGC. Several viruses also have been implicated in the etiology of SGC[6]. The most common is Epstein-Barr virus (EBV) infection which has been implicated in the pathogenesis of certain subtypes of salivary gland tumors[7]. Another virus that has been linked to salivary gland tumors is the human papilloma virus (HPV) 16 and 18[6].

Cancer is still one of the prime causes of human morbidity and mortality, and 90% of all cancer deaths arise from metastasis formation. Of all the processes involved in tumor progression, local invasion and the formation of tumor metastases are clinically the most relevant but least well understood at the molecular level, and represent one of the great challenges in exploratory cancer research[8].

Yet, it is clinically important to determine whether a salivary gland tumor is benign or malignant preoperatively, because this information strongly influences the surgical procedure. Local excision or superficial parotidectomy is performed to treat benign tumors, whereas total parotidectomy with or without removal of facial nerve tissue is performed to treat malignant tumors[9,10].

Pleomorphic adenoma

Pleomorphic adenomas of the salivary glands (PAs) are the most common neoplasm affecting the salivary glands. They are considered biologically benign. Occasionally, the epithelial component, in isolation or in conjunction with stroma, undergoes malignant transformation, giving rise to carcinoma ex pleomorphic adenoma or carcinosarcoma. These are both aggressive malignant tumors[10]. Rarely, a Pleomorphic adenoma that is seemingly benign at the microscopic level will metastasize like a true carcinoma in that case it is referred to as, metastasizing mixed tumor (MZMT) or metastasizing pleomorphic adenoma (MPA). The most recent World Health Organization classification of salivary gland neoplasms categorizes an MPA as a malignant epithelial neoplasm and defines it as "a histologically benign pleomorphic adenoma that inexplicably manifests local or distant metastasis"[11].The metastatic spread is usually preceded by multiple episodes of local tumor recurrence[12].

Usually, PA arises in parotid glands where it represents 65% of parotid gland neoplasms. Even though no specific age range has been observed, PA is most commonly diagnosed in middle-aged patients. A bimodal distribution of patients stratified according to age with a two-peak incidence, at 24 and 51 years, respectively, has been demonstrated. PA is more common in females, with a male-to-female ratio ranging from 1:3 to 1:4. The clinical presentation of this neoplasm is characterized by a painless, slowly growing, firm mass. In the early phase of development, PA is usually movable, but following growth, the tumor becomes more nodular and more stable. Recurrent PA is

multimodal and appears as small nodules that may seem fixed on palpation. With adequate surgical excision, the prognosis is excellent[13].

In recent years, ***Czader et al***[13] have shown that PA is a genetically heterogeneous neoplasm characterized by different chromosome aberrations, translocations, and gene mutations. Different PA subgroups have been identified, with anomalies mainly involving the chromosomes 3, 9, and 12[13], while, ***Martinelli et al***[14] suggest that Pleomorphic adenoma gene 1 (PLAG1) is consistently rearranged in pleomorphic adenomas and is activated by chromosomal translocations involving 8q12, the chromosome region that is most frequently affected in these tumors. These researchers are in favor of the theory, which postulates that the myoepithelial cell, evolves into the varied somatic cell phenotypes present in pleomorphic adenoma, and reinforces the role of PLAG1 on the tumorgenesis of benign and malignant pleomorphic adenoma[14], but it should be noted that approximately 30% of PA does not show chromosome alterations[13].

Histologically; Pleomorphic adenoma is distinguished by its cytomorphological and architectural diversity[15]. The histological analysis is based on the differentiation of the epithelial cells and the amount and nature of the stroma. About 30% of Pleomorphic adenomas are classified as cellular (cell-rich) type of Pleomorphic adenomas. In this subtype the epithelial cells are arranged in solid strands or small ductal structures surrounded by sparse stroma. 20% of Pleomorphic adenomas are classified as the classic or the balanced type there was a balanced amount of epithelial and myoepithelial cells

and the stroma component. About 50% of Pleomorphic adenomas are classified as stroma-rich type of pleomorphic adenomas. There, the mesenchymal-like tissue predominated, often accounting for more than 80% of the total tumor volume[16]. The marked stromal variety observed in this neoplasm results from factors such as the quantity and distribution of proteins of the matrix produced by epithelial and myoepithelial tumor cells, corresponding to the myxoid, chondroid, osteoid and hyaline areas[15].

Glycosaminoglycans, which appeared to be hyaluronic acid and chondroitin sulphate, were demonstrated rarely in lumina, often between epithelial cells forming the matrix of myxoid tissue and, together with collagen, forming the chondroid tissue[17]. Metaplastic changes are observed frequently in sections[18]. Almost all Pleomorphic adenomas have focally thin capsules with abnormalities such as satellite nodules or pseudopodia. The myxoid subtype shows focal absence of capsule with the tumor cells merging into the surrounding normal salivary gland tissue[17]. Therefore, enucleation or local dissection of the pleomorphic adenoma is not a sufficient surgical treatment for this special tumor entity[19].

Researchers recommend, depending on the location of the tumor, a lateral or total parotidectomy as the treatment of choice. The most common complications of its surgical removal are facial nerve dysfunction (temporary or permanent) and auriculotemporal syndrome (Frey's syndrome)[19,20].

Mucoepidermoid carcinoma

Mucoepidermoid carcinoma is a common malignant salivary gland tumor. It appears around the fifth decade of life, being unusual in children under 10 years[21]. ***Deepti et al*** [22], found that the majority of the cases involved major salivary glands, 89.6% involved the parotid, 8.4% submandibular and 0.4% the sublingual glands. The palate was the most common site for minor salivary gland involvement[22].

Kofi et al[23], believe that Mucoepidermoid carcinoma (MEC) of the salivary gland arises from pluripotent reserve cells of the excretory ducts that are capable of differentiating into squamous, columnar, and mucous cells[23]. ***Xing et al***[24], suggest that myoepithelial cells are responsible for the development of MEC[24].

The term mucoepidermoid is used to define a distinct salivary gland tumor characterized by a mixed pattern of the following two main cell types: epidermoid and mucus-producing cells. However, a third cell type, the intermediate cell, which is not mucous or fully epidermoid, is often present. Intermediate cells are thought to be capable of differentiating into mucous or epidermoid cells. Because of this cellular heterogeneity, the histologic composition, biological behavior, and clinical course of MEC varies greatly. The remarkable variability in the biological behavior of these tumors resulted in different opinions about the appropriate classification, grading, and treatment[23].

Although some authors[22], classify MEC into low- and high-grade types, others[23] favor a 3-tier system that includes an

intermediate grade[23]. Prognosis is dependent on the clinical stage, site, grading, and adequacy of surgery[25,26].

MECs may be circumscribed and variably capsulated or infiltrative and fixed; the latter characteristics generally apply to higher-grade tumors. Most tumors are smaller than 4 cm in diameter[26].

MEC cells form sheets, islands, duct-like structures, and cysts of various sizes. The cysts may be lined with intermediate, mucous, or epidermoid cells, and they are filled with mucin. Papillary processes may extend into the cyst lumina, and this is occasionally a conspicuous feature. The intermediate cells frequently predominate[26]. The mucinous cells are large with distinct borders and have a foamy cytoplasm. The squamous cells may show large nuclei with prominent nucleoli and they are arranged in nests or solid areas in conjunction with the mucinous cells. The intermediate cells are round to oval and basaloid with scant pink cytoplasm and show no particular differentiating characteristics[27].

Low grade MEC appear under low power microscopy as a well-circumscribed mass with cystic areas containing mucinous material .Well-differentiated mucinous cells predominate. High grade MEC is more solid and has a more infiltrative pattern of growth. The tumor cells in high-grade pattern show marked nuclear pleomorphism and increased mitotic activity. Intermediate and epidermoid type tumor cells predominate in high grade MEC. Intermediate grade is characterized by solidly growing areas of epidermoid cells or intermediate cells with the latter cell types predominating[28].

Cytokines have been implicated where there is stimulation of tumor growth, For example, transforming growth factor beta-1 (TGF-beta 1) is found consistently with high-grade MEC[23].

Clinically, the tumor usually forms as a painless, fixed, slowly growing swelling of widely varying duration that sometimes goes through a phase of accelerated growth immediately before clinical presentation. Symptoms include tenderness, dysphagia, and trismus. Intraoral tumors are often bluish-red and fluctuant, and they may resemble mucoceles or vascular lesions. They occasionally invade the underlying bone[27].

MECs must be distinguished from necrotizing sialometaplasia, chronic sialadenitis, cystadenoma, cystadenocarcinoma, squamous cell carcinoma, epithelial-myoepithelial carcinoma, clear cell carcinoma (not otherwise specified), and metastatic tumor[28].

Distant metastasis and lymph-node metastasis were reported in up to 35% of cases with MEC. Lymph node and distant metastases are associated significantly with high grade MEC. Lethal clinical outcome is caused by uncontrolled locoregional disease and metastases to the lung, bone, and brain. Low grade MEC has a low malignant potential. Though researchers[26,28] emphasize that low and intermediate grade MEC may make unexpected distant metastases[26,28].

The treatment of choice is surgical excision with or without neck dissection. Radiotherapy is generally palliative in advanced tumors; it has little impact on prognosis[26].

Hyaluronan

The salivary glands like other tissues are made up of cells and extracellular matrix. The ECM consists of a variety of substances, of which collagen fibrils and proteoglycans are truly ubiquitous. In addition to the proteoglycans (PG), the hydrophilic ECM includes a variety of other proteins such as non-collagen glycoproteins[29].

Hyaluronan is one of the ECM components. Hyaluronan (HA) is a ubiquitous glycosaminoglycan composed of an unbranched linear chain of repeating D-glucuronic acid and N-acetyl-D-glucosamine disaccharide units, which commonly reaches a size of four MDa. Unlike other glycosaminoglycans, hyaluronan is never covalently attached to a protein core or modified by sulphation, yet, despite this simplicity of structure it has a wide range of functions[30]. New roles for hyaluronan have been discovered challenging the older, outmoded view that HA is simply a passive bystander; it now appears that HA is an active regulator of many dynamic cellular processes[31].

Hyaluronan is abundant in many tissues and has a structural role within the extracellular matrix because of its expanded coil conformation, which in solution can assume hydrated sphere morphology. This allows hyaluronan to create and fill extracellular space. It regulates cell movement and transport of extracellular components[30] by forming a continuous and porous biological meshwork that exhibits visco-elastic properties[32]. It influences the hydration[33] and physical properties of tissues, interacts with other extracellular matrix components associating non-covalently with proteoglycans such as aggrecan or versican to form large aggregates