Myoepithelial Cells (MEC) of the Salivary Glands in Health and Tumours

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ABSTRACT
Myoepithelial cells (MEC) are found in the secretory units of many mammalian exocrine glands such as mammary, sweat, lacrimal and salivary glands. They are interposed between the secretory cells and the basal lamina. Immunohistochemically they are found to contain keratin intermediate filaments and are, therefore, considered to have an epithelial origin but at the same time they contain a large number of myofilaments which represent a massive expression of contractile proteins such as actin, myosin, calponin and caldesmon. Thus have smooth muscle like property also and hence the name. Numerous functions of MEC have been described, the most important of them being important for contraction of the glands and recently it has been found to prevent tumour progression. It should be noted that the diversity in the occurrence and dilemma regarding the pathogenesis of salivary gland tumours is due to lack in uniformity regarding the cells participating in its oncogenesis, especially the MEC. Also proper and extensive studies regarding MEC are very limited and thus have posed difficulty for a pathologist to understand this cell. In this review we try to bring about a thorough description of this cell in both physiological and pathological aspects.

Keywords: Functions, Immunohistochemical markers, Myoepithelial cell expression in salivary gland tumours

INTRODUCTION
In many exocrine organs, the secretory end pieces and the ducts are partly covered by cells with long processes that form an interlacing network. These cells resemble smooth muscle cells in several important aspects, yet clearly are epithelial cells, and thus are referred to as myoepithelial cells (MEC) [1]. The MEC were first discovered in the breast tissue by Krause in 1865. Since then it has been observed in the terminal end pieces and ducts of most of the exocrine glands such as salivary, mammary, sweat, lacrimal and bronchial glands. The description of these cells is diverse. Various authors have described them as “spindle shaped cells”, “star shaped cells” or “basket cells” until the term “myoepithelial cells” was conferred to it. Most of the authors have studied this cell in detail in the mammary glands only until it was Tamarin in 1966 that provided a vivid image of a typical acinus-associated MEC in the salivary gland. He typically described the appearance of the cell on the acinar unit as being “like an octopus sitting on a rock”. These cells lie between the basal lamina of the acinar and ductal cells at the terminal portion of the salivary glands [1,2].

Positive identification of salivary gland MEC on routine microscopic preparations is very difficult. Fortunately modern sophisticated microscopic techniques have resulted in a surge of new information on this cell such as exposure of MEC by chemical removal of periacinar connective tissue and basement membrane deposits [2]. MEC have many cytoplasmic processes which embrace glandular cells. Their nuclei are localized in the cell body. Most of the cytoplasmic organelles are found in the small areas around the nucleus. The remainder of the cytoplasm is filled with filaments and vesicles which are morphologically similar to those found in smooth muscle cells [3].

After studying the structure of MEC in detail researchers focussed on identifying the physiological functions of these cells. The function of MEC was identified by an essential term in biologic kinetics (kinesiology) which states “form defines the function”. The shape of the MEC suggested that its contraction might reduce the luminal volume in glandular endpieces, and these cells may play a role in expelling secretory products from glandular endpieces to the excretory duct system which was later proved experimentally. Thus one of the chief functions of MEC was determined [4]. Association of tumours with MEC was determined when scientists found that a variety of tumours occurred in salivary glands and breast as compared to pancreas and concluded that this was because of presence of MEC in the former two glands [5,6] [Table/Fig-1].

S. No. | Year | Study Done | Author
--- | --- | --- | ---
1 | 1865 | Identification of MEC in breast tissue | Krause
2 | 1966 | MEC studied for the first time in a salivary gland | Tamarin
3 | 1971 | Study of role of MEC in salivary gland tumours | Hubner et al.,
4 | 1973 | Role of MEC in expulsion of saliva determined | Emmelin et al.,
5 | 1974 | Fluorescent property of MEC identified | Puchtler et al.,
6 | 1976 | Cytochemical analysis of MEC in a salivary gland | Han et al.,
7 | 1977 | Role of MEC in histogenesis of salivary gland tumours | Regezi et al.,
8 | 1986 | IHC antibodies against normal MEC identified | Darke et al.,
9 | 1986 | Silver staining of MEC identified | Lanzzel
10 | 1994 | Identification of actin as marker for NMEC | Araujo et al.,
11 | 1997 | Identification of myosin as marker for NMEC | Savera et al.,
12 | 1999 | Identification of calponin as marker for NMEC | Prasad et al.,
13 | 2004 | Identification of maspin as marker for NMEC | Rde et al.,
14 | 2011 | Identification of WT1 as marker for NMEC | Langman et al.,
15 | 2013 | Identification of role of EWSR1 in myoepithelial cell tumours | Shah et al.,

This review aims to describes the MEC in detail regarding its development, distribution, and function mainly in the salivary glands. It also emphasises its role in the development of various tumours arising from these glands and methods to identify this particular cell in physiology as well as in pathology.
Distribution and Ultrastructure

The MEC in the salivary glands are stellate or spiderlike, with a flattened nucleus scanty, perinuclear cytoplasm and long branching processes that embrace the secretory and duct cells. The configuration of MEC depends on its location. Those associated with acinus are multipolar. They consist of a central body and four to eight processes radiating from it. Each process subdivides to give rise to second and even third generation of branches. Thus the net effect is that the acinus is embraced by many processes of the MEC [1]. In the intercalated ducts the MEC have a more fusiform shape and are elongated with few short processes. The processes in the acini lie in ‘gutters’, hence the outline of the acini appears smooth but in the intercalated duct, the processes runs longitudinally on the surface creating a bulge [7].

Ultrastructurally, the MEC consists of two moieties, a filamentous, and a non-filamentous one. The filaments are arranged parallel and aggregated as bundle and are approximately 4 nm in diameter, resembling the myofilaments of smooth muscle. The non-filamentous portion of the MEC contains the nucleus and the organelles. The nucleus is usually flattened in the plane parallel to the basement membrane, and may be scalloped in outline. The Golgi apparatus and prominent centrioles are also situated close to the nucleus. Mitochondria are not abundant, and they are evenly distributed in the perikaryon and in the cell processes. The endoplasmic reticulum consists of a few small cisternae [7].

FUNCTIONS OF MEC

Numerous functions of the MEC have been elucidated in the literature which include contraction, propagation of neural stimuli, basement membrane production, transport of metabolites and a role in tumour suppression [1,8].

Contraction

Since their discovery, the principle role of MEC that has been considered is its ability to contract. It was found that MEC played a very minor role in the expulsion of saliva during a normal basal flow but played an important role when secretion was required at faster rates such as during stimulation [9].

Propagation of Neural Stimuli

Though not proved, propagation of nerve stimuli is considered one of the functions of MEC. The detection of cholinesterase cytochemically and the presence of gap junctions ultramicroscopically support the hypothesis that MEC have a role in propagation of neural stimuli [1].

Basement Membrane Production

MEC plays an important role not only in the production of proteins such as fibronectin, laminin and elastin in the basement membrane but also acts as a scaffold for the multiplication and differentiation of these proteins [2].

Transportation of Metabolites

This is a controversial function that has been attributed to the MEC. The facts supporting this are that the presence of basal infoldings, presence pinocytotic vesicles, positive staining for and an increased alkaline phosphatase and magnesium dependant adenosine triphosphatase (ATPase) activity. However, it should be noted that ATPase activity in MEC can also be due to the fact that its a contractile cell [1,2].

Role in Tumour Suppression

MEC appears to resist neoplastic transformation. It has been shown that in the neoplastic state, these cells have a lower proliferation than basal type epithelial cells and secretes substances that inhibit tissue angiogenesis, invasion and metastasis [10,11].

IDENTIFICATION OF MEC

This is one of the most important aspects of MEC to be known because over the years lot of controversy has been around for histological identification of MEC. This section describes the various techniques that can be employed to identify these cells in the normal salivary gland.

With hematoxylin and eosin staining, the myoepithelial cell appears to be small and fusiform, its long axis being parallel to the basement membrane. It takes up a much darker stain than the duc tal epithelium [12]. The various special stains used to identify MEC and the stains that demonstrate the property of birefringerence and fluroscence of myosin and actin filaments has been described in [Table/Fig-2] [13-15]. The introduction of immunohistochemistry (IHC) to pathologists saw a new era in identification of both MEC and neoplastic MEC (NMEC). The various markers that have been used to identify these cells have been tabulated in [Table/Fig-2] [16].

MEC IN SALIVARY GLAND PATHOLOGY

The role of MEC in salivary gland tumours is well established. It can be said that MEC has the capacity to differentiate into epithelial as well as the mesenchymal components in a tumour and these tumour cells have been termed the NMEC. The ability of NMEC to provide a mesenchymal component to salivary gland tumours can be due to fact that MEC exhibits features of smooth muscle [2]. Eversole and later, Regezi and Batsakis were the ones who helped to understand the importance of NMEC in salivary gland tumours in the bicellular theory. It has to be understood that the NMEC has the ability to provide a wide spectrum of cytological and extracellular matrix (ECM) differentiation. It can also produce different architectural patterns in a tumour. The variety of morphologies that a NMEC can differentiate into is,

1. Angulate/basaloid: These cells are small with a hyperchromatic nuclei and a faint eosinophilic cytoplasm.
2. Epitheloid cells: These cells are polygonal with a vesicular nuclei and ample cytoplasm.
3. Clear cells: These cells appear clear due to glycogen in their cytoplasm.
4. Spindle cells: These cells are elongated, fusiform with pale cytoplasm.
5. Plasmacytoid (hyaline cells): These cells have a bright eosinophilic cytoplasm with eccentric nucleus.

The varieties of ECM that can be produced by NMEC include myxoid, chondroid, myxochondroid, and fibrous, elastic and even osteoid type of ECM. Similarly, the variety of architectural patterns that NMEC can produce are

1. Myxoid pattern: due to production of abundant chondromyxoid matrix, the tumour cells are loosely and randomly distributed.
2. Solid (non-myxoid) pattern: Cells are arranged in the form of nests, sheets intervened by a hyalinized matrix.
3. Reticular pattern: Seen as an anastomozing pattern containing epitheloid-myoepithelial cells.
4. Microcystic/Pseudocystic pattern: Presence of varied sizes of loose cystic spaces formed by accumulation myxoid matrix within the nests of tumour cells.
5. Cribriform/pseudoglandular pattern in which clusters of epitheloid cell form cribriform structures and pseudolumen due to their myxoid matrix production [17].

NEOPLASMS WITH MEC DIFFERENTIATION

[TABLE/FIG-3]

Recently Nagao et al., have classified the tumours with and without MEC cell differentiation. According to them the benign tumours that exhibit MEC cell differentiation are pleomorphic adenoma, myoepithelioma and basal cell adenoma. They did not include
sebaceous adenoma and oncocytoma which were considered to contain a MEC component earlier [18]. Though, it has not been determined whether the MEC observed in the tumour tissue carries itself tumourigenic potential or functions as a supporter of tumour induction, understanding its differentiation is very important to elucidate the pathogenesis of various salivary gland tumours [19]. It has to be noted that the variety of cytological, ECM and architectural patterns produced by NMEC can occur alone or along with other tumour components and according to this, the lesions showing NMEC differentiation can be classified under two headings: i. Tumours with complete/predominant NMEC participation (benign and malignant), ii. Tumours with partial NMEC participation (benign and malignant).

**Tumours with Complete/Predominant NMEC Participation (BENIGN)**

**Pleomorphic Adenoma and Myoepithelioma:** Tumour cells seen in pleomorphic adenoma and myoepithelioma are termed NMEC and are polygonal, spindle, or plasma cell-like in shape, which are arranged as sheet-, clump-, or strand-structure, and are admixed with myxoid, chondroid or osseous components which as mentioned before produced by the NMEC which attain these properties by way of differentiation into mesenchymal type of cells [20,21].

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Identification Methods</th>
<th>Markers or stains used</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Special stains</td>
<td>Silver staining, tannic phosphomolybdic acid-dye [21,22]</td>
</tr>
<tr>
<td>2</td>
<td>Birefringence</td>
<td>Thiazine Red R, Levanol Brilliant Red BB</td>
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<tr>
<td>3</td>
<td>Fluorescence</td>
<td>Rhodamine-phalloidin, Nitrobenzoxadiazole-phalacidin</td>
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<tr>
<td>4</td>
<td>Enzyme Histochemistry</td>
<td>Alkaline phosphatase, Adenosine triphosphatase, Glycogen phosphorylase, Inosine diphosphatase</td>
</tr>
<tr>
<td>5</td>
<td>Immunohistochemistry</td>
<td>α-SMA, SMMHα, h-caldesmon, Basic calponin, CK14, CK5, CK17, α1β1 Integrin, Metallothionein, p63, CD29 and CD109.</td>
</tr>
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**Basal cell adenoma:** The involvement of MEC in the pathogenesis of this tumour has been very controversial as almost all the histological and electron microscopic studies have not revealed the presence of NMEC in these benign tumour. However, few studies done using IHC have revealed presence of the NMEC in these tumours and their location has been correlated with that of a normal MEC in the duct [22-24].

**Tumours with Complete/Predominant NMEC Participation (MALIGNANT)**

**Malignant myoepithelioma:** The range of cell types seen in benign myoepitheliomas includes epithelioid cells (the most frequent) often arranged in trabecular or pseudo-acinar structures with cleft-like spaces (sometimes appearing signet ring-like), vacuolated (sometimes lipoblast like), hyaline (plasmacytoid) and spindle to stellate. In most malignant myoepitheliomas one cell type predominates, but there is usually a minority component of other cell types. The nuclei of malignant myoepitheliomas may be relatively uniform, small to intermediate sized and composed of finely distributed chromatin, lacking obvious nucleoli, or there may be marked cytological atypia, with enlarged pleomorphic nuclei, showing chromatin clumping and large nucleol [22].

**Epithelial-Myoepithelial carcinoma:** The tumour has a distinctive histopathologic pattern with a proliferation of ductular structures. The inner cells of these ductules constitute the epithelial component whereas outer cell layer that surrounds the ductules is considered the clear cell myoepithelial component of EMEC [25].

**Adenoid cystic carcinoma:** A malignant tumour, in which the occurrence of MEC has been much disputed, is the adenoid-cystic carcinoma or cylindroma. Adenoid cystic carcinoma especially the cribriform variant is composed of epithelial and abluminal cells, the latter usually being much more abundant. Although the histological appearance is much more basoloid than myoepithelial, a proportion of the abluminal cells show ultrastructural and immunohistochemical evidence of myoepithelial differentiation. Also, the hyaline material seen is a result of the production by NMEC [6,26].

**Metastasizing pleomorphic adenoma and Carcinoma ex pleomorphic adenoma:** Metastasizing pleomorphic adenomas are a small percentage of pleomorphic adenomas that have obvious malignant components in epithelial or both epithelial and mesenchymal components that can metastasize. The presence of NMEC dominates in this tumour and also in carcinoma ex pleomorphic adenoma as suggested both histologically and immunohistochemically [22].

**Tumours with Partial NMEC Participation (MALIGNANT)**

**Basal cell adenocarcinoma:** It is a rare tumour which is differentiated from its benign counterpart from its ability to invade adjacent structures brought about by the NMEC component [27].

**Polymorphous low grade adenocarcinoma:** Few authors have found that expression of some immunohistochemical markers of NMEC has been seen in very few cases of PLGA thus speculating the participation of these cells in this malignant tumour [28].

**Markers for Identification of Neoplastic MEC**

Salivary gland tumours are characterized by a wide variety of histological types, which makes their classification and diagnosis difficult. This complexity has been attributed to the NMEC. However, it has not yet been established that they actually originate from MEC because they often lack epithelial and smooth muscle proteins that are normally expressed by MEC [29,30]. Identification of the NMEC cannot be achieved without knowing which proteins are expressed by developing, immature MECs. It is therefore important to determine what proteins might be specifically expressed - and those which are not expressed by the MEC so that an immunohistochemical profile of these cells can be established [2]. Also, identification of proteins expressed by these cells can be helpful in identifying the origin and also help in understanding the biology of the tumour [29].

The NMEC express numerous proteins as expressed by the MEC. They are α-SMA, SMMHα, CK14, p63 and calponin [31-40]. Apart from these proteins there are few proteins that have identified to identify NMEC. Vimentin that is not expressed by MEC is considered to be a very sensitive marker for NMEC. The reason that has been stated is it expressed in basal cell adenoma, ACC and in PLGA, but did not find its expression in tumours arising from the ducts or Warthin’s tumour, inverted ductal papilloma or mucoepidermoid
carcinoma. Hence, it has been theorised that vimentin is seen as one of the earliest differentiation markers for NMEC [35].

S100 protein, named as it is soluble in a saturated ammonium sulphate solution has also been useful to the label NMEC [30]. Its expression in MEC in normal salivary glands and NMEC in various salivary gland tumours has produced variable results. The NMEC in pleomorphic adenomas showed positivity but this discrepancy was attributed to the fact that there is a rich autonomic nerve supply to the acini and ducts. Hence it is not presently considered to be reliable markers for NMEC. Similar to S100 protein, glial fibrillary acidic protein (GFAP) has shown positivity in the ductal cells of the salivary glands rather than the MEC. But its expression in the NMEC in pleomorphic adenoma has shed new light in understanding the development of such tumours and can be asserted that these neoplastic cells are at a very early stage of differentiation and this marker has been used to differentiate between basal and NMEC along with S100, actin and calponin [41].

Maspin has been considered a reliable marker for NMEC of the breast, but its expression in salivary gland tumours has been very limited. But it has been used as a prognostic marker. As we have already discussed, MEC plays a role in tumour suppression. Few authors have studied its expression in salivary gland tumours and correlated with tumour invasion and have theorised that maspin might be an important prognostic marker as its expression is high in tumours that show minimal invasion [42,43]. Also, recently WT1 has shown to be a promising marker for NMEC as its expression in pleomorphic adenoma seems to correlate with the NMEC [44].

RECENT ADVANCES

Recently researchers have sought the relation between tumours of MEC arising from salivary glands (major and minor) and some of the MEC tumours arising in the soft tissues such as benign mixed tumour in the skin and myoepithelial tumour/parachordoma in the soft tissues. These soft tissues lesions have been found to have genetic aberrations in the PLAG1 (Pleomorphic adenoma gene 1), which is common alteration in pleomorphic adenoma and EWSR1 gene (Ewing sarcoma breakpoint region 1), in the soft tissue. Though initial reports suggest that myoepithelial neoplasms are a separate entity and cannot be included in subset of tumours arising from its soft tissue counterparts, further research in this direction with large sample size is required to say anything conclusively [45-49].

CONCLUSION

Numerous functions of MEC have been elucidated but the most important role as far as pathologists are concerned is its ability to suppress tumour formation and hence acts as a prognostic marker. These cells have been studied under varied conditions and various stains but still a proper IHC marker for either the normal or NMEC has not been found. Also the fact that these cells have complex make-up, the NMEC has the ability to take on any of several very different morphological forms, and another important aspect regarding the MEC is that it plays a significant role in tumour pathogenesis and understanding this cell in detail is required to comprehend the pathogenesis of numerous salivary gland tumours. Hence, proper research and techniques has to be applied to obtain significant knowledge regarding MEC and its role in salivary gland tumours.

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