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A closer look into the cellular and molecular biology of myoepithelial cells across various exocrine glands

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Abstract

Myoepithelial cells (MECs) are a unique subset of epithelial cells that possess several smooth muscle cell characteristics, such as a high number of actin-myosin filaments and the ability to contract. These cells are primarily located around the secretory cells of exocrine glands, including the salivary, mammary, lacrimal, and sweat glands. Their primary functions involve the construction of the basement membrane and help with secretion of gland products through contraction. So far, no comparative analysis of MECs in different exocrine glands had ever evaluated their differences. In this review, we took advantage of the various publicly available scRNAseq data from mouse exocrine glands to identify their shared and unique characteristics.

The aim of this review is to compare the role of MECs in maintaining healthy glandular function, their involvement in disease states, and their regenerative capacity, with a particular emphasis on the latest research findings in these areas.

Introduction:

Myoepithelial cells (MECs) were first observed in the parotid salivary glands of cats in the 1860s, where they were described as "star-shaped cells" and "basket cells." In 1898, Zimmerman introduced the term "myoepithelial cells" to refer to this distinct cell type [1] that have properties of both epithelial cells and smooth muscle cells. MECs are situated around the secretory units of exocrine glands, including acini and in some gland's ducts, between the basal lamina and secretory acinar/ductal cells. While acinar cells are responsible for exocrine product secretion, MECs contract to facilitate the expulsion of these products through the branched duct system.

Each MEC has small central part and possesses multiple long branches, called processes, that extend outward and wrap around secretory structures, connecting with neighboring cells [1, 2]. MECs interface specifically with acinar cells and other MECs through cadherin junctions and desmosomes. They also connect with neighboring myoepithelial cells through gap junctions, and with the basement membrane through hemi-desmosomes [3].

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MECs are served by both sympathetic and parasympathetic nerves, both of which can trigger contraction, while gap junctions between MECs may allow synchronization of these contractions [4]. In addition, we and others reported that MECs have high levels of plasticity and are able to transdifferentiate into other cell types upon gland injury [5-7].

Several publications have examined the roles of MECs in different glandular tissues, and their potential implications in various diseases [8-14]. However, it remains unclear to what extent MECs in different glands/organs exhibit unique structural or gene expression features. This review aims to address this question by analyzing MECs from various organs.

MEC function in healthy tissue

Across several exocrine gland types, MECs help secretion of fluids from secretory cells by contracting, which, like in muscle cells, is made possible by interactions between the smooth muscle actin (αSMA) and smooth muscle myosin expressed by MECs. Contraction can be initiated by nerve signals or hormones such as oxytocin [8, 15-17]. Since MECs are located around the acini and ducts, synchronized contraction reduces the volume and length of the acini and ducts, pushing the fluid out [3, 18].

Another major function of MECs is to synthesize components of the extracellular matrix (ECM) and basement membrane of exocrine glands. MECs secrete elastin, laminin, and fibronectin to create the basement membrane, as well as structural components of the ECM such as chondroitin sulfate proteoglycan. Through the synthesis of structural proteins, MECs are also involved in directing morphogenesis, especially in development of luminal cell polarity, which is important for the proper formation and function of ducts and acini [19-21]. A study of Gudjonsson and co-authors demonstrated that mammary gland luminal cells develop correct polarity and form acinar structures when cultured in a gel containing laminin but not in a collagen gel [21]. However, when co-cultured with MECs in a collagen gel, the luminal cells also develop correct polarity. This implies that certain signals originating from MECs play a crucial role in the polarization of acini [19]. Other studies have shown that removing desmosomal proteins from MECs disrupts both acinar development and positioning of MECs, suggesting that the desmosomes formed by MECs are also necessary for normal morphogenesis and gland maintenance [3].

In addition, MECs regulate exocrine gland morphogenesis by secreting soluble factors and signals. Thus during lacrimal gland (LG) and submandibular gland (SMG) development, MECs surround the distal part of the developing bud and secrete cytokines and growth factors, which encourage differentiation of acinar cells [1, 5, 22].

MECs are found in various exocrine glands where they play a crucial role in the secretion and expulsion of glandular products, maintaining epithelial polarity, and gland homeostasis.

Function of MECs in different glands

Mammary Glands—Mammary glands are exocrine glands that produce milk. In mammary glands, the secretory units are generally termed alveoli. Analogous to acini, alveoli empty into branched ducts leading out of the gland [18]. MECs are found in both the alveoli and ducts. Around the ducts, they form a regular monolayer (Fig. 1A,

white arrows) while around the alveoli, they form a looser discontinuous layer around the secretory cells (Fig 1A, red arrows). Mammary gland development occurs primarily after birth, and concludes by sexual maturity and lactation [18]. Physical stimulation of the nipples causes the pituitary gland to release oxytocin, which binds to receptors on the surface of MECs and initiates contraction to expel milk from the mammary glands. The number of oxytocin receptors increases during pregnancy and is greatest directly after birth [4, 18]. This process is the same in all mammals, but ruminants (suborder Ruminantia) have an additional milk storage structure called a cistern. A recent experiment in dairy cows showed that after stimulation with a combination of oxytocin and an oxytocin antagonist, there was a difference in fat content between freshly secreted and stored milk [23]. During milk secretion, a number of mammary secretory epithelial cells, mostly viable, are detached from the gland and leave with the milk. This exfoliation was increased in oxytocin induced fresh secretion, which suggests that it is caused by the mechanical force of MEC contraction [24]. During lactation the mammary epithelial cells, are constantly regenerating and being shed, which is a normal physiological process that occurs in lactating mammals [25]. Thus, MEC contraction during lactation is critical for the efficient removal of milk from the mammary gland and it helps mammary gland regeneration.

Sweat Glands—The eccrine sweat gland is the most ubiquitous sweat gland on the skin and regulates body temperature through sweat secretion. It is composed of a secretory coil formed by myoepithelial and secretory luminal cells and a duct tube. During sweat gland development, the epithelial attachments give rise to initial sweat ducts, which eventually undergo a process of central cell degeneration to create the lumen or interior space of the duct [26]. MECs are derived from the same cells as the glandular epithelium. These cells migrate to the basal layer of the epithelium and differentiate into MECs. Adult sweat gland comprises three distinct types of cells: clear cells, dark cells, and MECs [27].

In response to neural, hormonal, and/or mechanical signals, MECs contract to expel sweat from the gland [4]. In human apocrine sweet glands, MECs interact with nerves while secretory cells do not. It has been demonstrated experimentally that human apocrine sweat glands secrete sweat in response to mechanical, thermal, and chemical stimuli. This secretion coincides with movements in the gland ducts resembling “peristaltic waves” caused by MEC contraction, which move the sweat to the surface. This was considered evidence that contraction of MECs drives excretion from sweat glands [28]. Since epithelial cells in sweat glands contact the blood vessels through gaps in the myoepithelium, the contraction of MECs also modulates the composition of sweat by increasing or decreasing material transport to the secretory cells [4, 29]. During the process of sweat gland regeneration, MECs are believed to play a crucial role in the proliferation and differentiation of the epithelial cells that make up the gland. Studies have shown that myoepithelial cells can produce growth factors and cytokines that promote cell proliferation and migration [30]. It has been also shown that label retaining cells (LRCs) with myoepithelial characteristics are present in the acinar region of sweat glands [31].

Harderian gland—The Harderian gland is found in the majority of terrestrial vertebrates. This multifunctional gland located within the eye's orbit in many vertebrates is the largest

structure in some species, such as mice and dogs [32]. In some animals, like birds and reptiles, the Harderian gland primarily produces tears to keep the eyes lubricated and moist. In addition, the Harderian gland secretion can contain pheromones assisting in communication and social interactions [33]. There are only 2 types of secretory cells in Harderian glands: secreting lipid droplets (A cells) and cells containing dark granules of multilamellar bodies (B cells) [34, 35]. The Harderian gland also secretes the triglyceride analog 1-alkyl-2,3-diacylglycerol, porphyrin [32, 36]. Similar to other exocrine glands harderian gland has MEC [37-39] (Fig. 1B). Interestingly, the density of MECs in the hamster Harderian gland is higher in female glands than in male glands [40]. It has been reported that MECs of the rat Harderian gland can also contract and promote secretion by acinar cells [41]. In the study conducted by Del Cacho and colleagues [42], it was noted that MEC cells within the chicken Harderian gland exhibited the potential for transdifferentiation into myofibroblasts. Nevertheless, this intriguing observation lacked further experimental validation.

In conclusion, recent publications mainly provide information about MEC morphology [43] and thus, the field would benefit from an in-depth characterization of their functions in the Harderian gland.

Salivary Glands—Major salivary glands consist of the submandibular, sublingual, and parotid glands. These glands each secrete different components of saliva, which protect the teeth and soft tissues of the mouth [44]. Both myoepithelial and acinar cells in salivary glands are served by sympathetic and parasympathetic nerves, which together initiate MEC contraction [45]. Kawabe and coauthors reported that MEC processes are thick, short, and simply branched in the sublingual gland, which secretes mucous saliva, whereas in the SMG, that secretes both serous and mucous saliva, the MECs processes were thin, long, and complexly branched [46] (Fig. 1C-D). Moreover the intensity of secretion is related to morphological changes in the MECs [47].

Song et al. performed lineage tracing analysis to map the cell fate of MECs [48]. The data were confirmed by scRNA-seq, which revealed a cluster of MECs that expressed contractile genes such as *Acta2*, *Myh11*, and *Myl9*, in addition to basal ductal markers like *Krt14* and *Krt5*, as well as *Sox10*, which is expressed by progenitors forming acini, MECs, and intercalated ducts [49]. Altogether, this suggests that MECs in salivary glands may represent a mixed cell lineage or an intermediate cell population. Expression of markers of both acinar and ductal lineages may also suggest a transdifferentiation ability of these cells [50]. Yuki Shindo's research team conducted a groundbreaking study on salivary gland development and regeneration [51]. α-SMA expression was observed in endbud epithelial cells, and its pattern of expression closely resembled that of muscarinic acetylcholine receptor M1. In addition they demonstrated that the stimulation of rat embryonic submandibular glands with acetylcholine or carbachol (an acetylcholine agonist) promotes the gland development and differentiation of MECs [51]. Moreover treatment with pirenzepine, a specific antagonist of M1 receptors resulted in complete abrogation of *Acta2* gene expression [51]. These findings highlight the important role of neural mediators in differentiation of MECs. It has been also reported that nerve growth factor (NGF) induces expression of MEC genes during

development. In addition the NGF and glial-derived neurotrophic factor (GDNF) family play significant roles in salivary gland healing after injury [52].

In addition, adrenaline and neural stimulation applied to differentiated MEC induces contraction and causes faster secretion from the salivary gland [47]. It was reported that MECs could be stimulated separately from acinar cells with the protein bradykinin [53]. Moreover, when MEC contraction has been induced it resulted in a much higher pressure and faster saliva flow in the duct system. These results suggest that passive secretion from secretory cells provide a background level of saliva while MEC contraction provides larger amounts in an acute manner when needed during the ingestion of food, for example.

Lacrimal Glands (LG)—The LG is a gland that produces aqueous component of tears to lubricate and protect the ocular surface [54]. MECs are also present in the lacrimal gland and play a role in the contraction and secretion of glandular products [8, 55] (Fig. 1E-F). Lacrimal gland development starts at E13.5 and is induced by FGF10 expressed by mesenchymal fibroblasts [54, 56]. As LG development progresses, the outer layer of cells within the LG bud acquires αSMA expression [5]. However, these cells remain devoid of any processes and retain an epithelial cell-like appearance (Fig. 1E). Single cell RNA sequencing of embryonic LGs and MEC lineage tracing also suggest that establishment of MEC lineage happens early in LG development [5, 22, 57]. In adult LG, MECs have long processes that physically interact to form a contractile network within the acinar part of the gland (Fig. 1F).

The establishment of myoepithelial lineage in the mouse LG is a complex process that involves the interplay of various signaling pathways and transcription factors. FGF10, SOX9 and SOX10 signaling plays a critical role in the establishment of myoepithelial lineage in the lacrimal gland [5, 58, 59]. FGF10 has been shown to regulate the expression of *Sox9* in the lacrimal gland, which is required for the expression of *Sox10* during lacrimal gland formation [60]. SOX10 is also an important transcription factor involved in the development and differentiation of MECs. Thus, *Sox10* mutants exhibit lacrimal gland hypoplasia leading to formation of the lacrimal gland branches missing differentiated acinar and MECs [59]. Interestingly, while *Sox10*+ cells have the potential to differentiate into vascular mural cells in various tissues [61], this congenital *Sox10* mutation has been shown to selectively impact the development of MECs and acinar cells without affecting pericytes and vascular smooth muscle cells that are present in the LG [59]. This indicates that certain types of pericyte/smooth muscle cells in distinct organs, like in the LG, may not necessarily rely on *Sox10*. Finally, contrary to acinar cells, most MECs of the LG maintain SOX10 expression in mature adult mice [62], thus further supporting their capacity for self-maintenance throughout mouse life [5].

MECs secrete various components including the lacrimal gland basement membrane and growth factors such as FGF2 [63]. Moreover, adult MECs in the murine and human LGs express various neurotransmitter receptors, including muscarinic receptors, which are activated by acetylcholine [55]. In particular, MECs of rat LGs express M3 muscarinic receptors, which are also found on the surface of LG acinar cells [64]. Activation of M3 receptors on acinar cells leads to an increase in intracellular [Ca^{2+}], which triggers

exocytosis of secretory vesicles into the lumen, while activation of M3 in MECs induces cell contraction to facilitate the release of secretory products [65]. Overall, the expression of neurotransmitter receptors on MECs in the lacrimal gland suggests that these cells play an important role in coordinating gland secretion in response to nerve signals, and that they may be a target for the development of therapies for dry eye syndrome and other lacrimal gland disorders.

MECs also can respond to cholinergic agonists [66, 67]. It has also been shown that in response to parasympathetic and sympathetic neurotransmitters, purinergic agonists and high potassium chloride in the lacrimal MECs the amount of intracellular Ca^{2+} increases and subsequent cell contraction occurs [67]. Similar to mammary glands, MECs in the lacrimal glands exhibit contractile behavior in response to oxytocin. In fact, our study conducted in 2018 confirmed the presence of oxytocin receptors in lacrimal MECs and demonstrated that artificial administration of oxytocin resulted in a significant decrease in gland acini size [68].

In conclusion, MECs in different exocrine glands have similar morphology; they typically have stellate (star-like) or spindle-like shape and form a layer or network around glandular acini (secretory units) or ducts. Moreover, MEC contractile nature and close association with glandular acini and ducts make them essential components for the proper functioning of exocrine glands. They help maintain glandular function and are critical for processes like secretion of saliva or tears, thermoregulation (sweat glands), and lactation. While there are certain common features that define MECs across different organs, there can also be unique characteristics based on their specific anatomical location and function.

Comparative analysis of MECs in various exocrine glands highlights shared patterns of gene expression, suggesting similar functions.

To investigate the differences and similarities between MECs of different organs, we mined publicly available scRNAseq dataset for tissues displaying a secretory function: SMG (GSE175649 and GSE150327) [69, 70], mammary gland (Tabula Muris) [71], liver (Tabula Muris) [71], pancreas (GSE84133) [72], prostate (GSE146811) [73], and our LG dataset (GSE232146) [62]. All of these datasets were integrated together using the reciprocal PCA (RPCA) method [74]. This approach is well suited when large proportions of cells are non-overlapping across datasets. Then, Principal Component Analysis (PCA) and Uniform Manifold Approximation and Projection (UMAP) algorithm were used for dimensionality reduction and visualization. Following unsupervised clustering, we identified a cluster labeled by *Acta2* and *Epcam*, consistent with MEC identity. Consistent with previous reports, this MEC cluster was absent in pancreas and liver datasets. In the prostate, we found cells clustered together with MECs (*Acta2*$^{+}$/ *Epcam*$^{+}$ cells) from salivary/lacrimal / mammary glands; however, these cells were not MECs (Fig. S1A-C) as previously stated by immunohistochemical studies [75, 76]. Further analysis of this prostate cluster indeed revealed two subpopulations of cells (Fig. S1D).

The subpopulation 1 expressed *Acta2* but was negative for epithelial markers, such as *Epcam, Krt5, Krt14 and Krt18.* This subpopulation also expressed the mesenchymal marker *Vim,* and multiple markers of fibroblasts such *Col1a1, Col1a1, Tpm2* and *Sfrp2* suggesting that these cells are myofibroblasts (Fig. S1D). In the prostate, myofibroblasts

could participate in the reciprocal interactions between epithelial cells and stroma which are necessary to maintain homeostasis of normal prostate [77]. During tissue injury, activated myofibroblasts migrate to the site of injury and help in tissue repair by secreting various growth factors, cytokines, and ECM components [78]. So, the fact that myofibroblasts from the prostate cluster together with the MECs from other organs could be explained by the fact that these cells share similar transcriptional programs for common functions including the contractile activity, ECM remodeling and the maintenance of a niche.

The subpopulation 2 (Fig. S1D) was labeled with epithelial markers (*Krt5, Krt14 and Krt18*) but did not express *Acta2*. This subpopulation also expressed *Trp63* (classical basal ductal cell marker in pancreas, mammary, salivary and lacrimal glands) [79-81], suggesting that the subpopulation 2 most likely represented basal ductal cells. In the prostate, these cells represent a pool of cells that participate in tissue development and maintain the homeostasis of the gland [82].

Therefore, we excluded liver, pancreas and prostate datasets from further analysis and reanalyzed data for mammary, SMG, and LG to study the transcriptome of MECs (Fig. 2A). After integration of the three datasets, the major cell types in all three glands were identified (Fig. 2A), including cluster of MECs that co-expressed *Acta2* and *Epcam* (Fig. 2B, red arrows). Then, we identified the gene markers of MECs in each tissue independently and compared these gene lists to reveal the shared and unique markers between MECs from the different organs. The distribution of these genes is illustrated by the Venn Diagram and Table 1 (Fig. 2C, Table 1). We thus obtained a list of 69 genes significantly conserved between mammary gland, SMG and LG MECs (Table 1). Moreover, 76 gene markers were specific to LG; 106 genes were specific to SMG, and 366 genes were specific to mammary gland. The lists of all genes and their distribution are provided in Table 1.

Pathway enrichment analysis was conducted using Metascape to define the biological processes universally enriched in MECs compared to other cells (Fig. 2C). As expected, the most significant processes were related to cell motility and cytoskeleton dynamics, namely “supramolecular fiber organization” (25 genes) and “positive regulation of cell migration” (14 genes). The most conserved MECs markers across tissues (FC>1.5, expressed by at least 70% of MECs from each gland) can be considered as a universal MEC signature, shown in Fig. 2D. In addition to genes related to motility and contraction (*Acta2, Myl9, Tpm2, Talgn, Tpm1, Myl6, Cald1*), MECs also expressed cytokeratin *Krt14*, the regulator of ion pumps and channels *Fxyd3*, the lipid carrier *Apoe,* the smooth muscle marker *Csrp1*, the modulator of myogenic differentiation *Igfbp5*, and surprisingly, some immunoregulators: cathepsin-L (*Ctsl*) and the antiviral protein interferon-induced transmembrane protein-3 (*Ifitm3*).To predict possible differences in cell function between MECs of each tissue, we submitted the entire markers sets from the three glands (including the 69 common markers) to Metascape (Fig. 2E).

Consistent with previous findings [55], we determined several pathways related to cell motility and muscle identity significantly enriched in all MECs. Interestingly, several processes related to cell proliferation and differentiation as well as developmental pathways were significantly enriched in MECs markers (Fig. 2E). This may suggest that MECs in

all three glands may undergo renewal and/or retain certain level of plasticity as we showed previously for LG MECs [5]. To further characterize the potential specificities in MEC establishment and identity across tissues, we studied the transcription factors involved in the cluster of pathways entitled ‘tissue morphogenesis’ including pathways like ‘morphogenesis of an epithelium’, ‘gland development’ and ‘morphogenesis of a branching epithelium’ (Fig. 2F). We thus found that, at the RNA level, few transcription factors were universally enriched in MECs of all glands, such as *Cebpb* that has promitotic effect on many cell types. For example, *Trp63* controlling epithelial morphogenesis and maintenance of stem cell populations [83], and a master regulator of eye and LG development *Pax6* [56, 84, 85] were more specific to the lacrimal MECs. By contrast, the regulator of stemness and differentiation *Kdm1a* was mostly detected in the mammary MECs. Salivary MECs expressed higher levels of *Tead1,* the activator of the evolutionarily conserved Hippo signaling pathway that controls organ size by regulating cell proliferation, apoptosis, and stem cell self-renewal [86, 87]. Moreover, the pathways “translational initiation” and “ribonucleoprotein complex biogenesis” were specifically enriched only in the mammary MECs, suggesting they may have a higher level of activity in protein synthesis (Fig. 2E). By contrast, “response to growth factor” including factors for cell-matrix and cell-cell interactions were significantly enriched only in the MECs of the LG and SMG (Fig. 2E).

We previously reported that in the LG, in addition to the acinar cells and other MECs, MECs interact with non-epithelial cell types and express many communication proteins [62]. The MEC network is indeed intricately embedded in the vascular system of the LG (Fig. 3, Supplementary movies 1-3). Using the *Acta2*-GFP reporter mouse labeling star-shaped MECs and mural cells wrapped around blood vessels stained with CD31, numerous physical connections can be observed between MECs and vascular cells (Fig. 3A). These interactions happen between MECs mural cells as well as endothelial cells, of both arterioles (Fig. 3B,C) and capillaries (Fig. 3D). The extended processes of MECs may even connect different blood vessels (Fig. 3D). Moreover, the cell bodies of some MECs may also interact with the blood vessel (Fig. 3E). To predict all interactions involving MECs in the three tissues (MG, LG, SMG), we used CellChat [88], with a threshold at 20% for the proportion of cells expressing ligands and receptors in sending and receiving clusters, respectively. Communication probabilities indicated that within exocrine glands, MECs exhibit a remarkable propensity to both receiving and transmitting signals to and from other cell types found in the datasets (Fig. S3A-C).

We found that, in all tissues, myeloid cells are predicted as the most probable receivers of MEC’s signals (Fig. 4A, Fig. S2D-F) mainly through APP/CD74 interaction, extracellular matrix (ECM) molecules (collagen, laminin, thrombospondin) and MIF signaling (in SMG and mammary gland). These ECM proteins likely affect multiple cell types, including MECs themselves (Fig. 4A). According to predictions, MECs also universally communicate with endothelial cells through VEGF signaling (Fig. 4A), by expressing *Pgf* in the LG/SMG and *Vegfa* in the MG. We noticed that in the LG and SMG, some ligands were expressed in over 50% of MECs, but their pathways were not included in the above analysis because their receptors were expressed in subpopulations smaller than 20% of main clusters (Fig. S2G-H). Thus, *Kitl* is detected in 68% of lacrimal MECs and signals to *Kit*+ epithelial progenitors-like cells of the intercalated ducts [62]. In the SMG, MEC-derived *Csf1* likely

attracts macrophage and monocytes expressing *Csf1r* (Fig. S3H). Salivary MECs also highly expressed *Postn*, which promotes epithelial adhesion and migration. *Postn* was predicted to be engaged in autocrine signaling (Fig. S3H) and may signal to immune cells, fibroblasts, and even MECs themselves.

Major signals received by MECs originate from fibroblasts (Fig. 4B-D). Other main senders include endothelial cells, MECs and pericytes. Most of the pathways involve ECM-related molecules (mostly collagens, laminin, THBS), but also the growth factor *Mdk.* It is noteworthy that mammary MECs could be influenced by the production of *Angptl4*, which plays a regulatory role in lipoprotein metabolism, through both autocrine and paracrine signaling with vascular cells (Fig. 4D).

In summary, genes related to the contractile function of MECs are highly conserved across all three tissues. According to the mRNA expression levels, mammary gland MECs may possess a higher translational activity. Cell communication analysis showed that in all tissues, MECs are predicted to interact with many other cell types, particularly vascular cells, fibroblasts and myeloid cells, through ECM molecules and growth factors. Lastly, while numerous transcription factors might carry comparable importance during embryonic development, the adult MECs within each gland could maintain their expression in distinct ways. Consequently, their regenerative potential could vary, influenced by the specific tissue context to which they belong.

MEC function during injury and disease

Acute injury and regeneration—Due to epithelial cells exposure to environmental factors, viruses and bacteria, they are prone to damage and even death. As a result, epithelial tissues, including exocrine glands, have very high regenerative ability.

The regeneration of each epithelial cell type (basal, myoepithelial, luminal) in exocrine glands is still a topic of debate. Interestingly, it appears that matured ductal luminal and MECs, which are mostly lineage-restricted during homeostasis, have a high level of plasticity after injury. These cells can possibly be activated through dedifferentiation or redifferentiation to repair damaged tissue, based on their tissue compartment and proximity to the wound [5, 89]. For example, Lu and co-authors found that MECs and luminal cells regenerated from unipotent sources when selectively ablated with toxins in a single sweat gland, remaining unipotent during regeneration [90]. However, in the mammary gland, MECs transplanted into mouse fat pads were able to generate polarized gland-like structures, including luminal cells that differentiated from the transplanted MECs [90]. Our research group has demonstrated that after interleukin-1α (IL-1α) injury, the MECs located in the LG are capable to transdifferentiate replacing damaged acinar cells [5]. Similarly, it has been shown that in salivary glands over 80% of regenerated acini originate from mature cells, such as MECs and ductal cells, which acquire a progenitor-like state prior to differentiation into acinar cells [91].

Another study has shown that MECs of submucosal glands have the ability to proliferate, migrate, and differentiate into different cell types in the airway surface epithelium following injury [92]. The study found that during regeneration, MECs in the submucosal gland

and trachea surface epithelium express the transcription factor SOX9, which play a crucial role in this regeneration process. Inhibition of SOX9 resulted in a significant decrease in regeneration, indicating that it is a key component of cellular plasticity [92]. These findings suggest that MECs in certain exocrine glands exhibit a remarkable level of plasticity. This also suggests that although epithelial cells are typically considered lineage restricted by epigenetics after early development, they can be activated and differentiated through certain conditions such as injury or culturing *in vitro*. Characterizing the specific physical or chemical triggers that lead to this plasticity could have important implications for regenerative medicine applications, including the potential for facilitating tissue and organ repair through injected cells in epithelia and exocrine glands.

Chronic Inflammation—Sjogren's syndrome (SS) is a type of autoimmune disease that affects several exocrine glands (including LGs and salivary glands), leading to gland dysfunction, inflammation, and immune cell infiltration.

According to a study conducted by Kapsogeorgou and coauthors [93], intercellular adhesion molecule 1 (ICAM1), a cell surface protein, is significantly elevated in salivary gland biopsy samples from human SS patients, suggesting its involvement in the development of SS. ICAM1 is known to play a key role in the activation of T cells [93, 94]. In addition, the expression of ICAM1 was significantly higher in MECs [93]. Notably, this pattern was observed even in cells far away from the infiltration sites [93]. These findings suggest that the abnormal phenotype of MECs in the gland precedes immune cell infiltration, rather than the other way around [93]. Another study used a lupus-prone sialadenitis mouse model [95] and found that ruptures in the basement membrane and death of MECs were associated with the development of autoimmune disease in the salivary gland. Electron microscope images of tissue sections revealed that this coincided with the attachment of lymphoid cells to MECs, which may be mediated by ICAM1, suggesting direct mechanical means of destruction [95]. Thus, the immune response may be triggered by abnormal production of cytokines and epithelial cell death [96, 97], but further research is needed to confirm this and determine the exact mechanisms of MEC destruction.

In a recent study utilizing two genetically modified mouse models of SS, we investigated the alteration of secretory function and MEC contraction in the lacrimal glands [8]. The study found that these models of chronic LG inflammation had smaller size of MECs, lower count of oxytocin receptors, and reduced oxytocin-induced secretion compared to wild-type mice [8]. Additionally, the key proteins involved in MEC contraction were suppressed by inflammation at some point after transcription [8], indicating that the proteins themselves, rather than gene expression, were being affected by the inflammation [8]. More recently, it was shown that MECs may directly contribute to mouse LG inflammation by activating the AIM2 inflammasome and cGAS/STING pathways in response to circulating self-genomic DNA associated with SS pathogenesis [98].

Thus, MECs appear to play a crucial role in both the initiation of SS and its effects on secretion. A more in-depth study of the precise mechanisms involved would undoubtedly lead to important insights into the prevention and treatment of SS.

Neoplasia and Cancer.: In 1997 Sternlicht and colleagues [12, 99] compiled evidence demonstrating that MECs possess inherent tumor-prevention properties. Specifically, MECs secrete basement membrane structure and proteinases that provide physical and chemical resistance to tumors. Sirka and colleagues [100] have provided further evidence supporting the tumor suppressing role of MECs by demonstrating their remarkable ability to restrain invasive cancer cells. Time-lapse imaging has revealed that MECs can actively capture migrating out epithelial cells in real-time, preventing their escape [100]. Furthermore, these studies have demonstrated that when MECs are organized into organoids alongside activated luminal cells, a greater number of MECs results in more effective control over cell migration [100]. This discovery is of great importance as it indicates that a compromised myoepithelium, as observed in inflammatory diseases, could elevate the likelihood of cancer metastasis.

Despite the importance of MECs, they have been less studied in cancer research [101] compared to luminal cells, which are responsible for the majority of breast cancers [102, 103]. Furthermore, tumors that contain MECs are more likely to be benign or low risk and tend to secrete more extracellular matrix (ECM) material, as opposed to degrading it as seen in many malignant tumors [3, 104]. Thus, MECs are not typically the initiators of tumors, likely because of their slower rate of proliferation compared to luminal cells and their inherent ability to promote cell adhesion and repress cancer cell migration. However, one study in mammary gland [105] identified a notable deviation from the tumor suppression trend. Indeed, tumor associated MECs in ductal carcinoma tissue were found to stimulate the progression to invasive carcinoma by expressing TGFβ, which may enhance cancer cell plasticity. This was confirmed through cocultivation of ductal carcinoma cells with MECs obtained from tumors [105]. Furthermore, several types of rare salivary gland neoplasms and cancers are linked to MECs. Cancers arising from MECs are known to progress rapidly and lethally [106], suggesting that MECs partially lose their differentiation. The neoplasia of MECs is strongly correlated with reduced level of their differentiation within the tissue [107]. Some well-known tumors of salivary glands involving MECs with varying degrees of malignancy are pleomorphic adenoma (most common), myoepithelial carcinoma, epithelial-myoepithelial carcinoma, adenoid cystic carcinoma, and mucoepidermoid carcinoma [1, 108-113]. Besides that, within many mammary gland tumors that contain MECs, these cells had the ability to differentiate into multiple phenotypes, resulting in different structures and extracellular matrix (ECM) deposition patterns. In some carcinomas of the mammary gland, MECs exhibit an altered phenotype and fail to produce laminin 1, which is necessary for the proper polarization of luminal cells [114]. This suggests that matrix molecules secreted by MECs play a crucial role in suppressing tumors, and their loss may contribute to tumor initiation.

Thus, the role of MECs in cancer progression is ambiguous and strongly depends on the histological context and oncogenic drivers. In normal, healthy tissue, MECs act as tumor suppressors. However, disruptions in tissue homeostasis, such as mutations or interactions with nearby tumor cells, can trigger pathways that lead MECs to promote cancer progression by losing their typical morphology and ability to produce ECM molecules.

Determining the MEC function using organoid cultures

Organoid cultures are a powerful tool for studying the functions of different cell types. In particular, organoid cultures containing myoepithelial cells (MECs) have been extensively utilized in studies, notably in research related to the mammary gland. Three-dimensional (3D) mammary organoid cultures have indeed become a crucial tool in the field of mammary gland biology, studies of mammary gland development and disease in a physiologically relevant and controlled environment, leading to valuable insights and breakthroughs in the field [115-119]. Thus upon stimulation the organoids were able to generate milk production and had normal histological structure including a fully operational contractile myoepithelial layer [115]. Another study analyzed factors maintaining organoid cell growth, proliferation and differentiation [117]. This study unveiled a novel role for progestin in fostering MEC proliferation. Specifically, it demonstrated that progestin induces the expression and secretion of nuclear factor-κB ligand (RANKL) by luminal cells. Moreover specific interaction of RANKL with hepatocyte growth factor significantly amplifies MEC proliferation[117]. In a study conducted by Sirka and co-authors [120], the dissemination potential of various epithelial cell types, including MECs, was assessed. The authors also examined the interactions between luminal and myoepithelial cells using inducible Ubiquitous-*Twist1*, Myoepithelial-*Twist1*, and Luminal-*Twist1* organoid cultures. These models enabled the isolation of the myoepithelium's role in *Twist1*-induced dissemination. Specifically, the expression of *Twist1* in MECs resulted in the appearance of disseminated cells expressing K14+, indicating that myoepithelial-specific *Twist1* expression led to cell-autonomous myoepithelial dissemination. Meanwhile, normal myoepithelium was found to dynamically restrain *Twist1*+ luminal and tumor cells. Furthermore, the authors demonstrated the existence of distinct populations within breast tumors: invasion-suppressing (K14+SMA+) myoepithelial cells and invasion-promoting (K14+SMA−) epithelial cells. This study [120] demonstrates that MECs can form a dynamic barrier to prevent luminal epithelial dissemination. It has been also shown that organoid cultures originating from luminal and myoepithelial cells produce organoids with lineage-specific restrictions [116].

Salivary and lacrimal gland organoids have also been utilized to study myoepithelial and other cell function. Thus, Yoon and coauthors recently established long-term murine and human salivary gland organoid cultures [121]. This study showed that murine and human salivary glands organoids maintain the cellular heterogeneity and structural diversity of different salivary glands [121]. Moreover, they also showed that functional unit that include secretory and myoepithelial cells similar to other *in vivo* studies could be stimulated with neurotransmitters, suggesting that salivary gland function under different conditions could be studied using organoids. Recently, patient-derived salivary gland organoids have been used to study salivary gland cancers [122].

Several studies showed that LG 3D organoid cultures can differentiate into the miniature glands containing several cell types including, myoepithelial and secreting-competent cells [123, 124]. Moreover differentiation of LG organoids required *Pax6* expression [124], which correlates with our previous finding [56].

Recently iPS cell-derived organoids have been extensively used to model diseases related to myoepithelial cells. For example, researchers have created organoid models to study conditions like salivary gland disorders and breast cancer, where myoepithelial cells play a crucial role [125, 126]. Using iPS derived organoids scientist can assess how myoepithelial cells respond to different drugs and therapies, potentially leading to the development of more effective treatments for glandular diseases [126]. Thus The iPSC-derived lacrimal gland organoid model holds great potential to study lacrimal gland development and morphogenesis [127]. By employing patient-derived iPSCs, it becomes possible to investigate the development of genetic lacrimal gland diseases, such as lacrimal and salivary gland aplasia and lacrimo-auriculo-dento-digital syndrome. iPS derived organoid cultures also have the potential to be used in personalized medicine approaches. For example, patient-derived iPS cells can be used to create organoids that closely resemble the patient's tissue, allowing for customized treatment strategies.

Myoepithelial cell plasticity

Several studies indicate that salivary and LG multipotent epithelial cells exist only during embryonic development, while in adult uninjured glands, cell lineages are restricted and do not give rise to other cell types [5, 57, 128]. Analysis of cultured human mammary gland myoepithelial and luminal cells obtained through reduction mammoplasty shows that these cell types maintain their characteristics in vitro [129]. This study indicates that neither cell type undergoes transdifferentiation during cultivation suggesting the cell lineage restriction in homeostatic mammary gland.

It has been widely reported that MECs of several exocrine glands show high level of plasticity. Thus high percentage of mammary gland basal MEC cells can form colonies and repopulate a mammary gland *in vivo,* suggesting that at least some of mammary MECs have stem cell properties [7]. This idea has been supported by several publications demonstrating that in various exocrine glands, MECs are long-lived, label-retaining cells [5, 7]. Notably, lineage tracing experiments conducted on injured salivary and LG revealed that cellular plasticity plays a significant role in the process of glands regeneration through the transdifferentiation of MECs [5, 6, 91, 130]. Thus, in both LG and salivary glands MEC and acinar lineages retain plasticity after maturation and can transdifferentiate into other cell types upon injury [5, 91, 130]. In various injury models, the airway surface epithelium is repopulated as a result of the proliferation and migration of submucosal gland MECs [92, 131, 132]. These findings suggest that following the damage, the plasticity mechanisms could be triggered to participate in tissue regeneration. The mechanism of cellular plasticity is not well known. However, in mammary gland p63 and NOTCH1 have been described as a master regulators of myoepithelial and luminal cell fate specification [133]. Thus, basal MEC marker p63 promotes basal fate specification, while NOTCH1 has an opposite role, forcing MECs to acquire a luminal fate [134]. Mammary glands undergo a recurring sequence of proliferation, differentiation, and involution during pregnancy, suggesting that mammary gland epithelial cells should retain a high level of plasticity, or these glands should have multipotent stem/progenitor cells [135]. Although it would be hard to determine whether a high level of plasticity or multipotent stem cells are involved in the process of mammary gland remodeling, since the downregulation of cell lineage-specific factors

(for example, P63 or NOTCH1) allows cells to acquire a less differentiated phenotype that could be indistinguishable from the stem cells. Additionally, one can consider the possibility that myoepithelial cells in the mammary gland may exhibit greater plasticity in contrast to MECs in the salivary and lacrimal glands. This enhanced plasticity could be linked to the significant transformations occurring during mammary gland remodeling. Significant role in cellular plasticity of exocrine glands plays stromal microenvironment that includes stromal cell types and extracellular matrix [136]. The extracellular matrix ensures the structural integrity of epithelial cells while also influencing biological processes such as cell differentiation, migration, and transformation into malignant cells [137]. As MECs in all exocrine glands both interact directly with the extracellular matrix and secrete certain extracellular matrix components [55], they could be more significantly influenced by extracellular matrix cues [138].

The Myoepithelial cells: an evolutionary perspective.—MECs, known for their contractile properties and often identified as specialized modified epithelial cells, constitute a distinct cell type with a remarkable evolutionary conservation. Myoepithelial cells can be found in a range of organisms, including invertebrates, reptiles, birds and mammals [42, 43, 139-142]. For example, colonial hydroids have mitochondrion-rich myoepithelial cells that contract and regulate the gastrovascular flow [142]. MECs play similar roles in different animal phyla and various organs. While the details of myoepithelial cell biology may vary between species, the fundamental function of facilitating glandular secretion or liquid propagation through contraction is a common feature.

Little is known about primary non-transformed human myoepithelial cells. However, according to the PanglaoDB database, 24 out of the 26 markers listed for MECs are common to both human and mouse species. While our review primarily focuses on MEC data derived from mouse models, many of the observations and conclusions drawn from these studies can thus be extrapolated to human MECs due to the high degree of conservation of these cells across species throughout evolution.

Conclusions

MECs were found in several exocrine glands and are crucial for maintaining tissue structure, function, and homeostasis in these glands. The scRNAseq analysis provided insight into a consistent observation: despite variances in MEC localization and morphology across different glands, their primary function remains contractile, associated with genes involved in cytoskeleton dynamics for cell motility. Beyond contraction and their physical interaction with secretory cells, MECs likely affect other cell types (vascular cells, immune cells) through the secretion of signaling factors and ECM molecules and thus, may regulate numerous processes at the tissue level. Lastly, mature MECs exhibit markers associated with cell proliferation and transcription factors implicated in tissue development. This observation implies a certain degree of plasticity and regenerative potential within these cells. This notion finds support in experimental evidence, which has demonstrated that MECs from certain tissues (including LG, SMG, mammary gland, sweet glands) have the capacity to undergo transdifferentiation into cell types belonging to other lineages after injury or in culture [5, 31, 67, 91, 143]. These findings suggest that MECs possess a level of

plasticity that enables them to respond dynamically to environmental signals and fulfill diverse cellular demands. Nevertheless, additional research concerning the regenerative capabilities of MECs would be necessary to uncover the extent of their cellular plasticity in each tissue.

In conclusion, while MECs share main fundamental features across different tissues, such as their contractile function and cytoskeletal dynamics, MECs also display a few tissue-specific attributes. The degree of tissue-specific behavior of MECs depends on factors like the microenvironment and local signaling cues allowing them to adopt tissue specific appearance and distinct roles in response to tissue-specific needs.

Limitations of the study:

In this review, the comparison of myoepithelial cells across exocrine glands is based on their transcriptional profiles determined by scRNA-seq. Consequently, some of the functions and interactions inferred from this data may require validation at the proteomic level and through functional studies. Furthermore, it is important to note that our analysis was limited to publicly available data and may not include information about myoepithelial cells (MECs) in certain tissues, such as the Harderian gland, or in different animal phyla. With the continuous growth in the number of single-cell atlases being made available to the scientific community, we view this analysis as preliminary and anticipate that the integration of additional datasets will lead to a more comprehensive description of MECs in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ANGPTL	Angiopoietin-like proteins
APP	Amyloid precursor protein
CDH	Cadherin
CSF	Colony stimulating factor
CXCL	chemokine (C-X-C motif) ligand
EPHA	Ephrin-A
FN1	Fibronectin 1

GAS	Growth arrest specific
HSPG	Heparan sulfate proteoglycan
MHC-I	Major histocompatibility class I
MIF	Macrophage migration inhibitory factor
MK	Midkine
PDGF	Platelet-derived growth factor
PTN	Periostin
THBS	Thrombospondin
VEGF	Vascular endothelial growth factor

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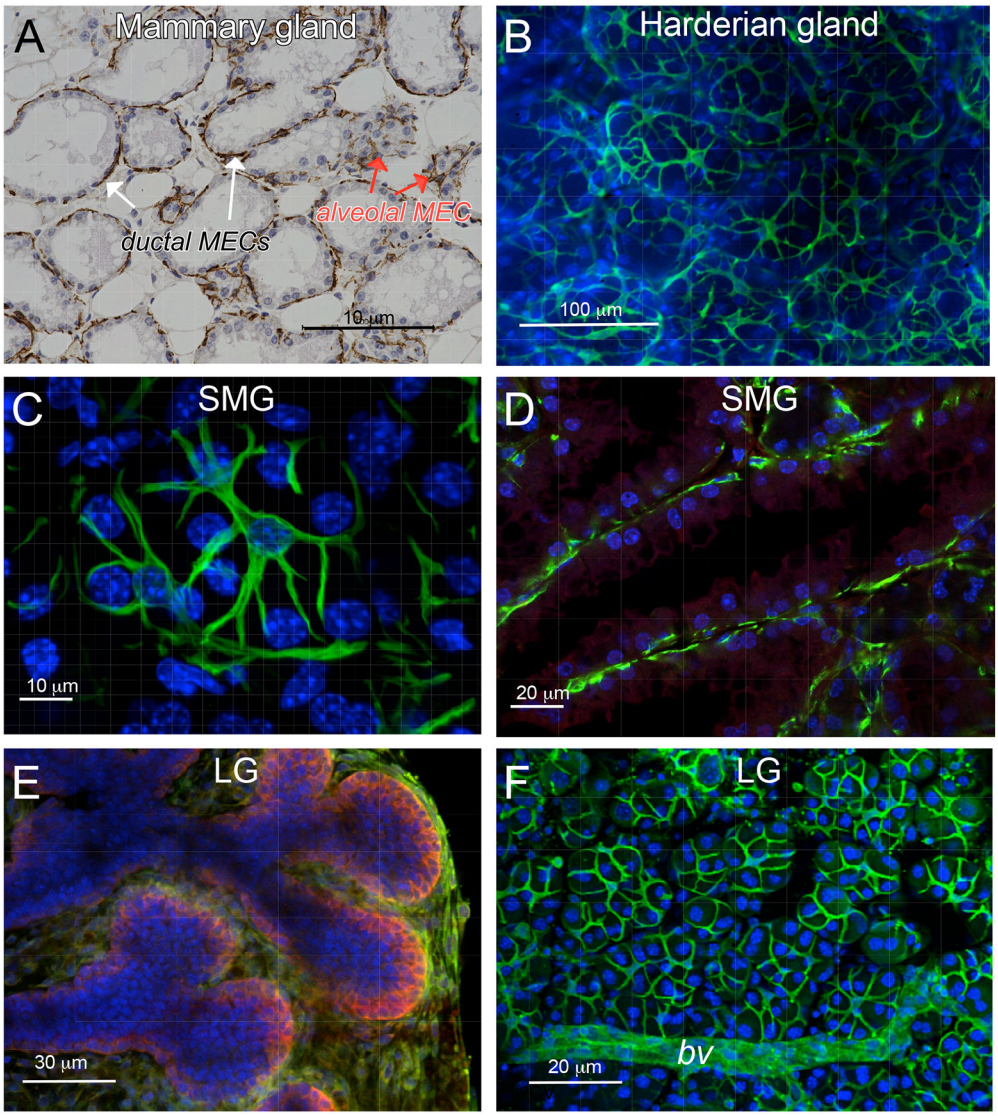

Figure 1: Illustration of MEC appearance across diverse exocrine glands.

(A) Immunostaining of paraffin section of a lactating mouse abdominal mammary gland (day 2 postpartum) with αSMA antibody reveals MECs around both ducts and alveoli. Around the ducts, MECs form a regular monolayer (white arrows) while around the alveoli, they form a looser discontinuous layer (red arrows). (B) Harderian gland (HG) MECs are located around acini. Whole mount immunostaining with αSMA antibody, nuclei are stained with DAPI (4′,6-diamidino-2-phenylindole). (C-D) MECs of SMG are located around acini (C) and ducts (D). MECs were detected by immunostaining with the αSMA antibody. Ductal secretory cells in (D) were stained with the antibody to LAMP1 protein. (E) During development LG initiates αSMA expression (red) in the outer layer of cells within the LG buds at E13-16. However, these cells are lacking any processes and retain an epithelial cell-like appearance. Mesenchymal cells were stained with the Vimentin antibody. Nuclei stained with DAPI. (F) Immunostaining of adult LG with the αSMA antibody reveals MECs

around LG acini. Antibody also labeled mural cells within blood vessels (*bv*). Nuclei stained with DAPI.

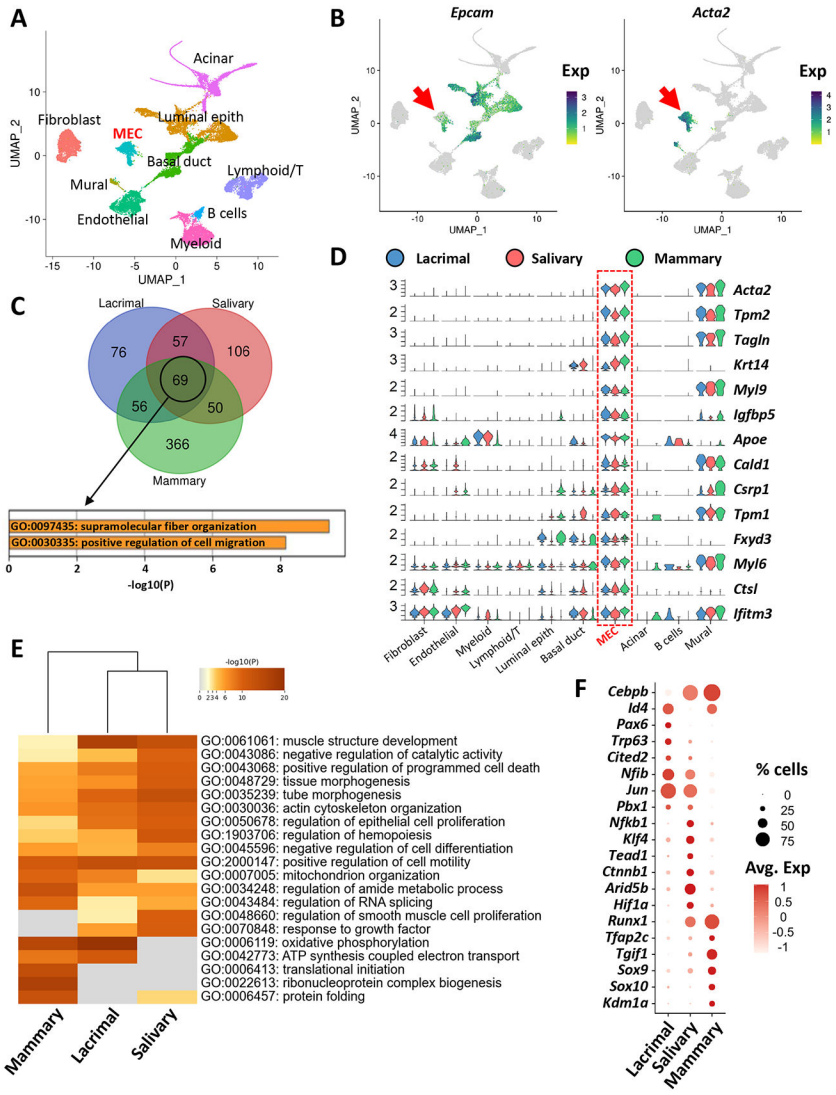

Figure 2: Single cell RNA-seq analysis of integrated datasets from submandibular (SMG), lacrimal (LG) and mammary (MG) glands.

(A-B) UMAP plot of integrated dataset from LG, SMG and MG. The cluster of MECs (red) was identified by the co-expression of *Epcam* and *Acta2*. (C) Venn diagram showing distribution of MEC markers between tissues. The 69 genes shared by the SMG, LG and MG are involved in cell contraction and cytoskeleton dynamics. (D) Violin plot showing the expression of the most conserved markers (FC>1.5, expressed by at least 70% of MEC) in all clusters. Violins are colored by tissue identity. (E) Clustered heatmap of pathways enriched in the three independent lists of MEC markers shows that most of them are highly conserved across tissues. (F) Dot-plot of transcription factors expressed by MECs respective to tissue type.

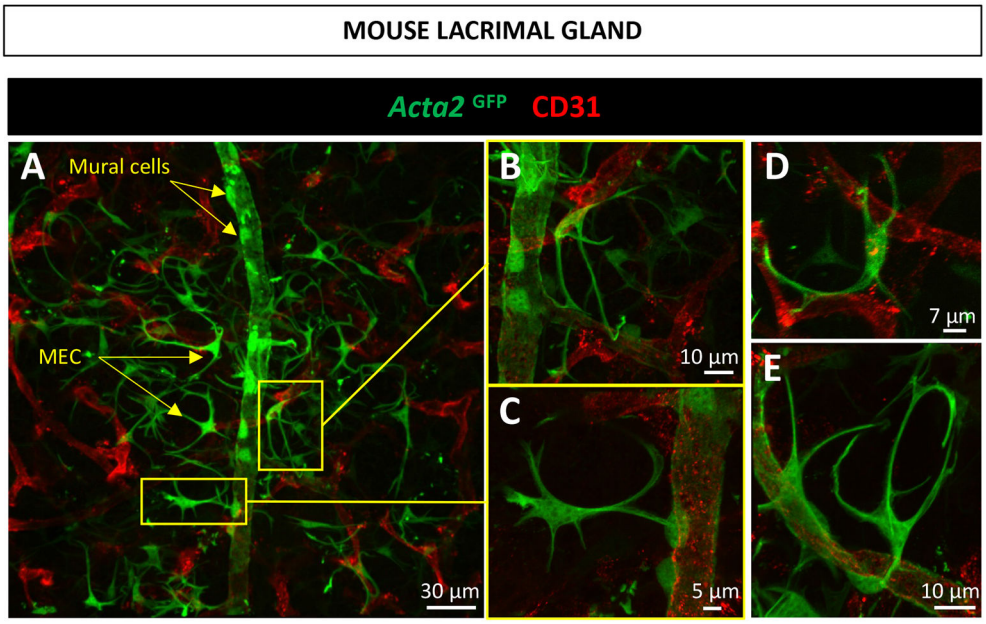

Figure 3: in the LG, MECs closely interact with vascular cells.

Confocal images of LG whole mounts from a two-months old *Acta2*-GFP mouse. GFP labeled SMA-expressing cells (green) while blood vessels were stained for CD31 (red). Projection of z-stacks were done using Imaris software. Yellow squares on (A) indicate areas that were magnified in (B) and (C). Thanks to their stellate shape and long processes, MECs are easily discriminated from mural cells that are wrapped around blood vessels.

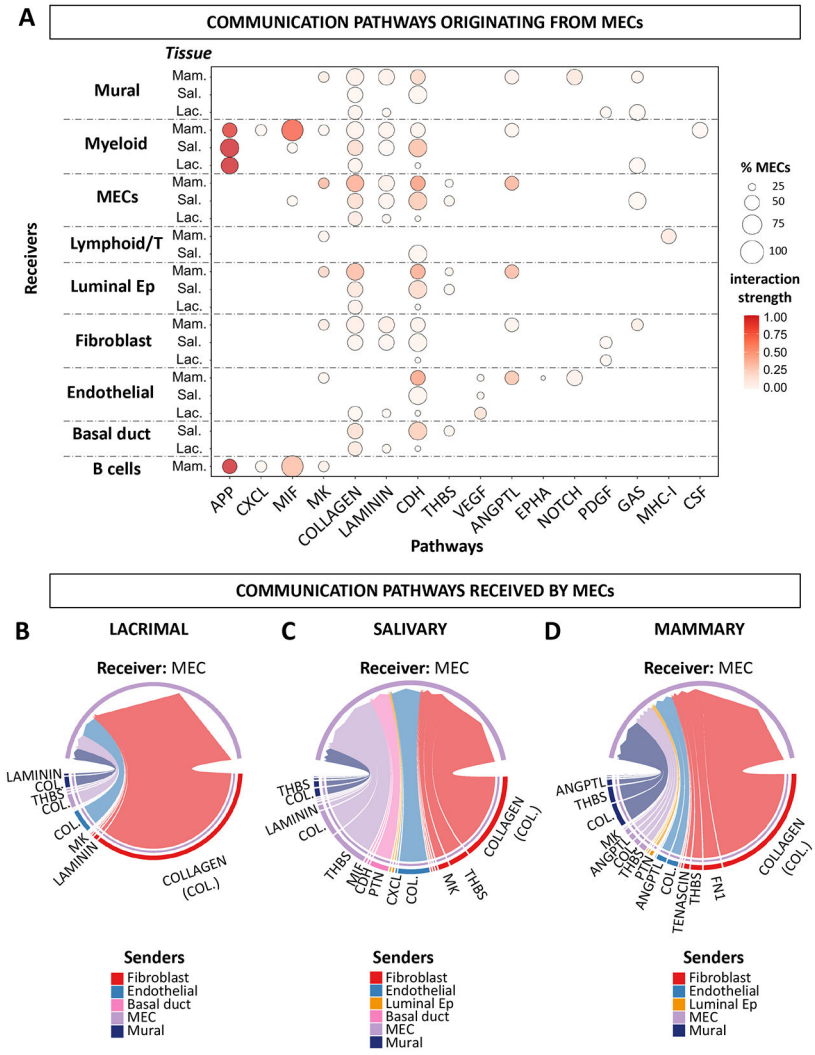

Figure 4: Analysis of MECs' outgoing and incoming signals.

(A) MECs' outgoing signals. Bubble plot showing all significant (permutation test, p-val <0.001) communication pathways originating from MECs in the mammary ("Mam."), the salivary ("Sal.") and the lacrimal ("Lac.") glands. Only ligand-receptor (L-R) pairs expressed in at least 20% of cells and clusters of at least 65 cells were considered for this analysis. Bubble size corresponds to the percentage of MECs expressing the corresponding ligand. If multiple ligands are involved in the communication pathway, the percentage of MECs expressing either of the ligands is shown. Bubble color correlates with the interaction strength (communication probability, red is high). For each gland, the interaction strength was normalized using min-max scaling of all MEC interactions in the corresponding tissue. (B-D) MECs' incoming signals. Chord diagrams summarizing the communication pathways received by MECs in each tissue. On the lower part of the chord diagrams, the inner thinner bar color represents the targets (MECs) that receive signal from the corresponding outer bar (see color legend below for cluster identity). The bar size is proportional to the signal

strength received by MECs. Communication pathways for L-R pairs involved are indicated next to their corresponding bar.

Table 1:

Venn Diagram distribution: List of markers for MEC per tissue

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
Krt17	Fosb	Tmed10	Tuba1a	Cox7c	Ehf	Ndufa5
Actn4	Mbnl1	Ppia	Maff	Gtf2h5	Dnajb1	Hspa5
Tsc22d1	Mia	Ndufb9	Runx1	Trim29	Luc7l2	Angptl4
Postn	S100a6	Edf1	Txnrd1	Rras	Foxp1	Lpgat1
Cald1	Kif5b	Atp5j	Odc1	Elob	Srpk2	Pabpc1
Krt5	Plcb4	Atp5e	Anxa6	Selenof	Map7d1	1700025G04Rik
Sdc4	Zfp36l1	Uqcr11	Sfn	Tgfbr3	1810037I17Rik	Rsl1d1
Nedd4	Rbms3	Myl12a	Sgk1	Cebpd	Gadd45b	Mdk
Itgb1	Smarca2	Hsp90ab1	Col4a2	Jund	Dcn	Akap2
Perp	AY036118	Eif1	Fus	Sparcl1	Midn	Cct3
Dynll1	Map1lc3b	S100a11	Tmem176b	Calm1	Ltbp4	Tgif1
Slc38a2	Lpp	Atp1a1	Capns1	G0s2	Hsph1	Strap
Dstn	Klf9	Nenf	Fgfr1	Atp5d	Ier2	Fermt1
Gapdh	Zbtb20	Cox7a2	Ptma	Cox4i1	Cdcp1	Tnfrsf12a
Myl9	Ppp1r12a	Serbp1	Taf1d	Ly6e	Gls	Col16a1
Ddx5	Nfib	Id4	Nhp2l1	Pgf	Nfkb1	Psma2
Atpif1	Itga6	Hnrnpk	Marcksl1	Ccnd2	Ythdc1	Lsr
Pebp1	Btg2	Phlda3	Sfr1	Selenok	Trib1	Snrpd1
Prss23	Ywhaz	Eef1d	Hnrnph1	Trp63	Capza2	Rhob
Igfbp5	Socs3	Cox6c	Icam1	Spon2	Nebl	Ndufa1
Rbp1	Cp	Cox7b	Sfpq	Neat1	Marcks	Hprt
Ifitm3	Gm42418	Uqcrb	Jup	mt-Nd4l	Gem	Metap2
Ddx3x	Tshz2	Atp5j2	Lmo4	Malat1	Chka	Ubxn4
Slc3a2	Fos	Map1lc3a	Gja1	Ndufa7	Eef2	Dusp6
Mt1	Sfrp1	Aldoa	Timp3	Krt8	Tmem176a	Arl4c
Fxyd3	Gas1	S100a10	Top1	Rhoj	Tmbim6	Tmem158
Zfp36l2	Pcp4	Ndufa4	Fst	Nrg1	Hk2	Cd24a
Hnrnpa2b1	Msrb1	Chchd2	Thbs1	Oaz1	Klf6	Rab18
Eif4g2	Oat	Atp5g1	Csf1	Rgs2	Ptp4a1	Psmb7
Tpm2	Purb	Psma7	Ets2	Cpe	Ckb	Nip7
Hspb1	Txnip	Phlda1	Clic4	Myh9	Ptrf	Mrto4
Palld	Nfix	Spint2	Tinagl1	Rpl10a	Adamts1	Ndrg1

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
Tpm1	Eif4a2	Mfge8	Rbms1	Atf3	Timp2	Atf4
Lcn2	Zyx	Bsg	1500015O10Rik	Slc6a6	Klf4	Dclk1
Lamb3	Egr1	Mif	Mt2	Cd164	Vim	Tomm20
Ier3	Kitl	Gpx4	Ctnna1	Lmod1	Nrip1	Tcp1
Atp2a2	Tns1	Srrm2	Nop58	Actb	Dst	Yrdc
Pfn1	Htra1	Uqcrq	Ube2s	Pfdn5	Itm2b	Hdgf
App	Gadd45g	Rps27l	Lhfp	Ifi27	Ctgf	1300014I06Rik
Ndufa11	Zfp36	Sumo2	Hnrnpu	Ntrk3	Tead1	Pfdn2
Flna	Dsp	Cox6b1	Ube2d3	Atp5h	Erbb2ip	Lgals7
Ctsl	Junb	Cox8a	Cdh1	Ndufa13	Fermt2	Synpo
Mylk	Pdgfa	Swi5	Serpinh1	Sem1	Sh3bgrl	Etf1
Hmgb1	Rabac1	Cd9	Eif1a	Cited2	Pam	Srxn1
Aplp2	Tax1bp1	Son	Amotl1	Matn2	Sepw1	Esf1
Krt14	Igfbp2	Cnbp	Mat2a	Actg1	Shroom3	Tagln2
Csnk1a1	Clstn1	Slc25a3	Lmna	Sema5a	Ets1	Baiap2
Cebpb	Meis2	Atp5b	Pmepa1	Rnase4	Meg3	Sox4
Hmgn1	Jun	Fabp5	Tuba1c	Grcc10	Nfat5	Npm3
Myh11	Nr4a1	Atp5f1	Tubb2a	Ccn1	Tgm2	Prdx1
Ptms	Pik3r1	Calm2		Epas1	Amotl2	Rnh1
Cnn1	Morf4l1	Ywhaq		Ndufb11	Kdm6b	Ddx21
Hsp90aa1	Sec62	Atp5a1		Selenow	Pkm	Ifi27l1
Acta2	Bgn	Atp5l		Tanc1	Mgll	Sdc1
Gas6	4930523C07Rik	Slc25a5		Atp5g2	Ktn1	Ddx24
Slc25a4	Col4a1	Actn1		Ccn2	Casp4	Tfg
Dbi	H3f3a			Tkt	Ppp1cb	Set
Csrp1				Fam129a	Ddr1	Psma3
Rbpms				Mme	Nfia	Ppa1
Pdlim4				Eid1	Lamp2	Tomm6
Myl6				Ndufa2	Cyr61	Nme1
Ifitm2				mt-Nd4	Wsb1	Sod2
Sparc				Naca	Irf1	Cct2
Laptm4a				Cav1	Emp2	Tubb6
Aldh2				Selenom	Arid5b	Slpi
Apoe				Pax6	Ski	Srsf6
Ubc				Cldn10	Pde4b	Lypd3

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
Lgals1				Nrtn	Sat1	Fosl1
Tagln				Abi3bp	Gstm1	Polr1d
				Tmem59	Hmox1	Cfl1
				Cck	Bag3	Rhoc
				Bri3	Ptbp1	Klf10
				Tppp3	Ngf	Morf4l2
				Lamp1	Brd2	Mia1
				Fbxo32	Hif1a	Pdap1
				mt-Nd5	Mast4	Psmc2
					Arl5b	Cstb
					Dynll2	Lmo1
					Pbx1	Ppib
					Azin1	Tpm4
					Csde1	Hsd17b10
					Hspa8	Mdh2
					Pkp4	Timm17a
					Steap4	Ppan
					Tln1	Rtn4
					Slc5a3	Snrpd2
					Hspa1b	Hsbp1
					Csrnp1	Nop2
					Psap	Hbegf
					Pnp	Trf
					Gnai2	Jag1
					Ier5	Eif3c
					Rassf1	Apoc1
					Atp13a3	Pdlim3
					Cxadr	1600029D21Rik
					Cryab	Epcam
					Stat3	Smtn
					Cltc	Vmp1
					Lama3	Mlf2
					Irf2bp2	Col9a2
					Ctnnb1	Ssr2
					BC005537	Cct8

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					Dnaja1	Kctd1
					Hspa1a	Ywhae
					2010111I01Rik	Mrfap1
					Tspan2	Plekhb1
						Baz1a
						Prmt1
						Por
						Pa2g4
						Ppig
						Grwd1
						Arc
						Eif5a
						Nedd8
						Tubb4b
						Nme2
						Map2k3
						C1qbp
						Gjb3
						Tmed9
						Lyar
						D11Wsu99e
						Zfand5
						Cox5a
						Gtpbp4
						Nudc
						Minos1
						Rn45s
						Gar1
						Llph
						Sox9
						Cd63
						Hnrnpc
						Rab6a
						Psmb6
						Tspan4

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
						Arf4
						Eif2s1
						Lamc2
						Denr
						Tceb1
						Mtap7d1
						Tmem93
						Selm
						Pkp1
						Plaur
						Rpl7l1
						Moxd1
						Tuba4a
						Eif3b
						Tm4sf1
						Efhd2
						Npm1
						Timm23
						Tspan3
						1110038B12Rik
						Col17a1
						Ran
						Psmb5
						S100a1
						Gjb4
						Calr
						Brix1
						Gch1
						Cdv3
						Clca2
						Cxcl14
						Rbbp7
						Taf9
						Tmem51
						Nop56

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
						Hnrnpab
						Hspa4
						Ppid
						Aqp3
						Chi3l1
						Errfi1
						Eif2s2
						Cct4
						Klf13
						Ybx1
						Eif3a
						Vcp
						Psmd7
						Txndc17
						Akr1a1
						Cpne8
						Mtap1b
						Oaz2
						Crispld2
						Eif4h
						2010002N04Rik
						Pqlc1
						Psmc6
						Tceb2
						Cd44
						1810011O10Rik
						Ebna1bp2
						Dusp7
						Pvrl1
						Bcam
						Spcs1
						Srsf2
						Ndufaf4
						Uchl3
						Crip2

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
						Dcun1d5
						Hspa9
						Srsf3
						Sox10
						Cox6a1
						Aprt
						Cyc1
						Mrpl52
						Wbp5
						Psmb4
						Timm10
						Txnl4a
						Ssb
						Dnaja2
						Itm2c
						Cd151
						Cox5b
						Ccrn4l
						Magoh
						Mthfd2
						Erh
						Sphk1
						Ncl
						Anp32b
						Tuba1b
						Nolc1
						Rrp1
						Blcap
						Krt15
						Ldha
						Rrs1
						Arf6
						Ptges3
						Eif3g
						Psmd12

Common markers between Lacrimal Mammary and Salivary Glands (n = 69)	Common markers between Lacrimal and Salivary Glands (n = 57)	Common markers between Lacrimal Mammary Glands (n = 56)	Common markers between Mammary and Salivary Glands (n = 50)	Specific markers for Lacrimal Gland (n = 76)	Specific markers for Salivary Gland (n = 106)	Specific markers for Mammary Gland (n = 366)
						Aebp1
						Psmb3
						Pgam1
						Nop10
						Hspd1
						Pdlim7
						Eif4a1
						Igfbp3
						Kdm1a
						Cxcl12
						Usmg5
						Pfdn4
						Cct5
						Taf13
						G3bp1
						Ywhag
						Gpatch4
						Tpbg
						Skp1a
						Timm9
						2900010M23Rik
						Ccdc86
						Fbl
						Cltb
						Timm13
						Srsf7
						Anxa2
						Tfap2c
						1190003J15Rik
						Fkbp4
						Slc2a1
						Nap1l1
						Eif6
						Tnfrsf1a
						Eif5

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						Mgp
						Cldn25
						Shfm1
						Ndufa12
						Ctsd
						Actg2
						Wnt10a
						Txn1
						Ndufb2
						Anxa5
						Efnb1
						Il17b
						Mrpl12
						Phb2
						Atox1
						Eif4ebp1
						Gtf2f2
						Syncrip
						Srsf5
						Gnl3
						Eif5b
						Psma5
						Pttg1ip
						Pdpn
						Emid1
						Rrbp1
						Mki67ip
						Ranbp1
						U2af1
						Atp5g3
						Polr2l
						Fhl2
						Vapa
						Chadl
						Snrpb

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						Cdc42ep3
						Timm8a1
						Gsto1
						Osgin1
						Eef1e1
						Cct7
						Tomm5
						Gng5
						Rbm8a
						Eny2
						S100a16
						Psmb2
						Ahnak
						Cycs
						Ltbp2
						Cnn3
						Luzp1
						Ddit4
						Ucp2
						Snhg1
						Tes
						Higd1a
						Pkm2
						Psma6
						Hnrnpf
						Serpinb5
						Ndufs6
						Sf3b4
						Hint1
						Chchd4
						Eif3d
						Cda
						1110008F13Rik
						Kcnq1ot1
						Srm

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						Ube2n
						Wwtr1
						Ywhah
						Nhp2
						Sepx1
						Nars
						Mtdh
						Cotl1
						Nfe2l2
						Ube2d2
						Romo1
						Eif4e
						Rheb
						Gpr56
						Pcbp1
						Rrp15
						Wdr43
						Pkp3
						Kdelr2