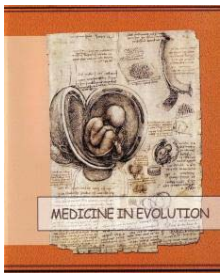
Myoepithelial cells of the lacrimal gland



Bâră R.I.[1,2], Petrea C.E.[3], Jianu A.M.[4], Oancea R.[5], Rusu M.C.[6*]

[1]PhD stud., Department of Ophtalmology, Faculty of Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Department of Ophtalmology, Bucharest University Emergency Hospital, Bucharest, Romania
[3]MD, PhD (Med), Division of Anatomy, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[4]MD, PhD, Dr.Hab., Department of Anatomy and Embyology, Faculty of Medicine, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania
[5]DMD, PhD, Department I, Faculty of Dental Medicine, "Victor Babes" University of Medicine and Pharmacy, Timisoara, Romania
[6]MD, PhD (Med.), PhD (Biol.), Dr.Hab., Prof., Division of Anatomy, Faculty of Dental Medicine, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Correspondence to:
Name: Rusu MC
Address: "Carol Davila" University of Medicine and Pharmacy, 8 Eroilor Sanitari Blvd., RO-050474, Bucharest, Romania
Phone: +40 722363705
E-mail address: anatomon@gmail.com

Abstract

Myoepithelial cells (MECs) are found in different exocrine glands, which include the lacrimal gland. However, although the MECs are functionally relevant for the function of this gland, few studies characterised them by means of immunohistochemistry. We therefore aimed at checking the molecular phenotype of the MECs in the lacrimal gland. For this study were used archived paraffin-embedded samples of lacrimal glands. Primary antibodies were applied: D2-40, alpha-smooth muscle actin (α-SMA), Ki67, the smooth muscle myosin heavy chain (SMM), cytokeratin 5 (CK5), and CD117/c-kit. MECs of the lacrimal gland expressed D2-40 (podoplanin), α-SMA, SMM, CK5 and, scarcely, c-kit. Ki67, the proliferative marker, and c-kit, were scarcely expressed in basal cells of the secretory units. Therefore, podoplanin should be added to the specific panel of markers specifically identifying the MECs of the lacrimal gland.

Keywords: podoplanin; immunohistochemistry; exocrine gland; orbit; markers.

INTRODUCTION

Myoepithelial cells (MECs) are found in multiple glandular organs such as the salivary, lacrimal, mammary, harderian, sweat, and prostate glands (Makarenkova and Dartt, 2015). However, the profile of MECs varies considerably from gland to gland (Nagato et al., 1980). The MECs of the exorbital lacrimal gland of rat are stellate with many thin radiating processes with tapered ends that terminate freely (Nagato et al., 1980).

The MECs characteristically present a stellate cell body with many ramified cytoplasmic processes lying on a basal membrane of acinar cells (Lemullois et al., 1996). They equally possess a cytokeratin network and a highly developed network of alpha-smooth muscle actin (α-SMA) (Lemullois et al., 1996). Therefore, the ultrastructural pattern of the MECs include cytoplasmic myofilaments which run parallel to the basal lamina and display focal densities along their course, pinocytotic vesicles and hemidesmosomes (Chaudhry et al., 1983).

In the lacrimal gland MECs synthesize the basement membrane and form a functional network around the acinar and ductal cells separating them from the basement membrane and the glandular stroma (Makarenkova and Dartt, 2015). The MECs maintain the glandular structural integrity and transport metabolites to secretory cells (Makarenkova and Dartt, 2015).

In spite of the proposed importance of MECs for the function of the lacrimal gland, they are lesser studied than in other exocrine glands (Makarenkova and Dartt, 2015). We therefore aimed at checking the molecular phenotype of the MECs in the lacrimal gland.

Aim and objectives

The aim of this study is checking the molecular phenotype of the MECs in the lacrimal gland.

MATERIAL AND METHODS

The immunohistochemical study was performed retrospectively on archived paraffin-embedded samples of lacrimal glands (six cases). The age of patients ranged from 49 to 57 years. The patients' written informed consent for all medical data to be used for research purposes, provided the protection of the identity is maintained, was obtained. The study was conducted in accordance with the general ethical principles of medical research.

The paraffin-embedded samples were processed with an automatic histoprocessor (Diapath, Martinengo, BG, Italy) with paraffin embedding. Sections were cut manually at 3 µm and mounted on SuperFrost® electrostatic slides for immunohistochemistry (Thermo Scientific, Menzel-Gläser, Braunschweig, Germany). Histological evaluations used 3 µm thick sections stained with hematoxylin and eosin. Internal negative controls resulted when the primary antibodies were not applied on slides.

There were used primary antibodies for D2-40 (clone D2-40, Biocare Medical, Concord, CA, USA, 1:100), alpha-smooth muscle actin (α-SMA, mouse monoclonal, clone D33, Biocare Medical, Concord, CA, USA, 1:50), Ki67 (mouse monoclonal, clone MM1, Biocare Medical Concord, CA, USA, 1:100 – 1:200), smooth muscle myosin heavy chain (SMM, mouse monoclonal, clone S131, Novocastra-Leica, Leica Biosystems Newcastle Ltd, Newcastle Upon Tyne, U.K., 1:100), cytokeratin 5 (CK5, rabbit monoclonal, clone EP42, Biocare Medical Concord, CA, USA, 1:100), CD117/c-kit (rabbit monoclonal, clone Y145, Biocare Medical, Concord, CA, USA, 1:100).

Tissues were deparaffinized and rehydrated, then endogenous peroxidase was blocked using Peroxidazed 1 (Biocare Medical, Concord, CA, USA). For the heat induced epitope retrieval we used the Decloaking Chamber (Biocare Medical, Concord, CA, USA) and

retrieval solution pH 6 (Biocare Medical, Concord, CA, USA). Background Blocker (Biocare Medical, Concord, CA, USA) was used to reduce non-specific background staining. The primary antibody was then applied. Different HRP-based detection systems were used: for α-SMA, Ki67, SMM, CK5, and D2-40 was used MACH 4 (Biocare Medical, Concord, CA, USA), and for CD117/c-kit was used MACH 2 (Biocare Medical, Concord, CA, USA). A HRP-compatible chromogen (DAB) was applied. Sections were counterstained with hematoxylin and rinsed with deionized water. For the washing steps we used TBS solution, pH 7.6.

RESULTS

The normal features of the human adult lacrimal gland were accurately identified on histological slides stained with hematoxylin and eosin. Myoepithelial cells expressed the smooth muscle myosin (**fig.1**). The α-smooth muscle actin was detected in MECs, as well as in pericytes and vascular smooth muscle cells (**fig.2**). CD117(c-kit) labelled myoid periendothelial cells, such as vascular smooth muscle cells and pericytes, as well as the MECs of the lacrimal gland (**fig.3**). Interestingly, expression of c-kit was also encountered in the membranes of basal acinar epithelial cells with a high nucleocytoplasmic ratio (**fig.3**). Octopus-like MECs also expressed cytokeratin 5 (**fig.4**) and D2-40 (podoplanin) (**fig.5**). Scarce basal acinar cells expressed Ki67 (**fig.6**).

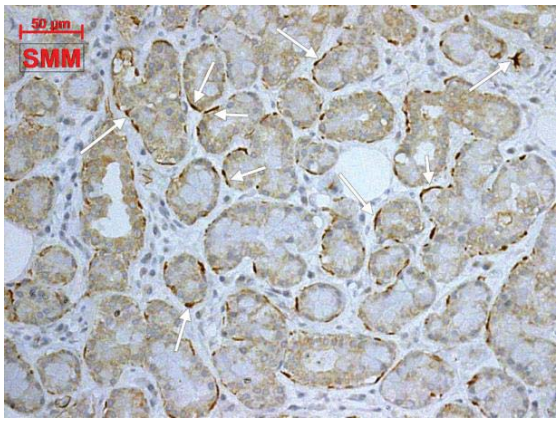


Figure 1. Immunohistochemical expression of myosin is detected in myoepithelial cells of the human adult lacrimal gland (arrows)

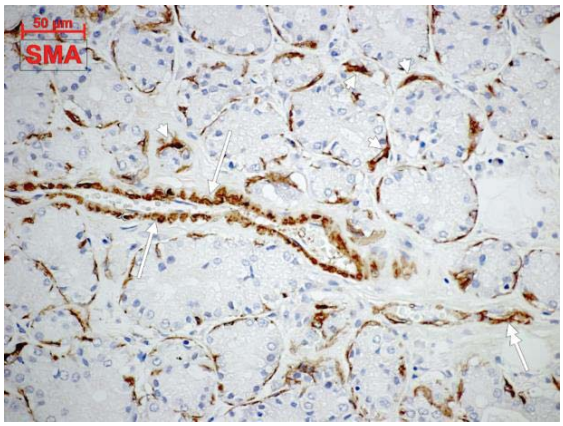


Figure 2. Immunohistochemical expression of α-smooth muscle actin is detected in myoepithelial cells (arrowheads), vascular smooth muscle cells (arrows), and pericytes (double-headed arrow) of the human adult lacrimal gland

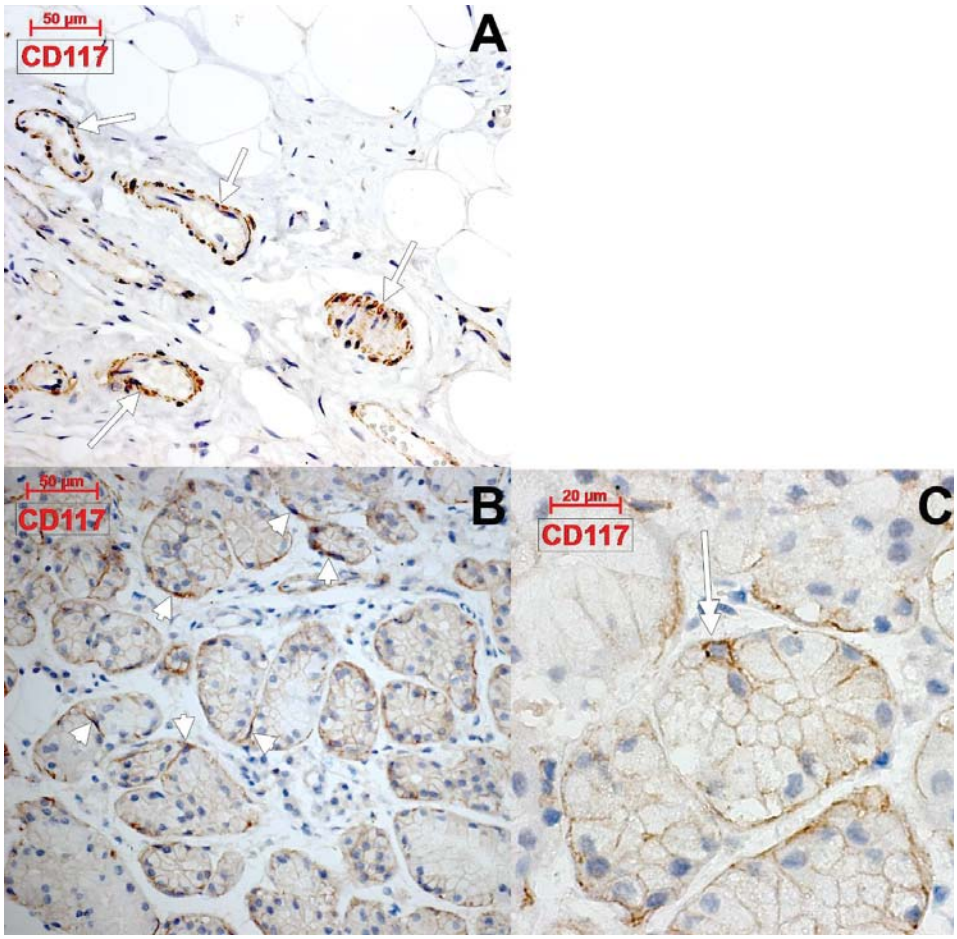


Figure 3. Immunohistochemical expression of CD117 (c-kit) was found in vascular smooth muscle cells (A, arrows), in myoepithelial cells (B, arrowheads), and, scarcely, in basal epithelial cells of the acini (C, double-headed arrow).

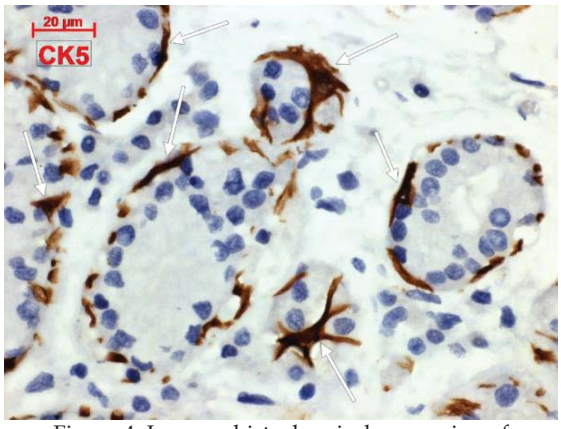


Figure 4. Immunohistochemical expression of cytokeratin 5 in myoepithelial cells (arrows) of the human adult lacrimal gland

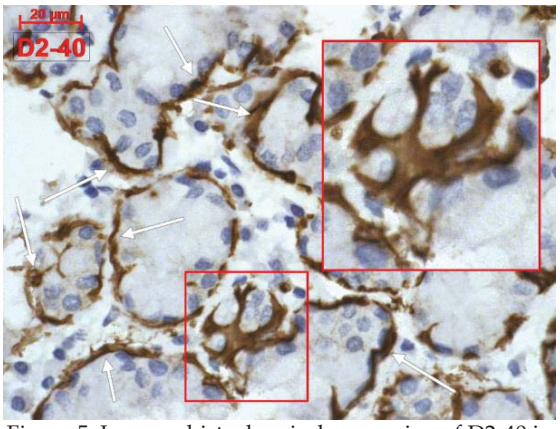


Figure 5. Immunohistochemical expression of D2-40 in myoepithelial cells (arrows) of the human adult lacrimal gland. The octopus-like appearance of the myoepithelial cells is detailed (magnified inset)

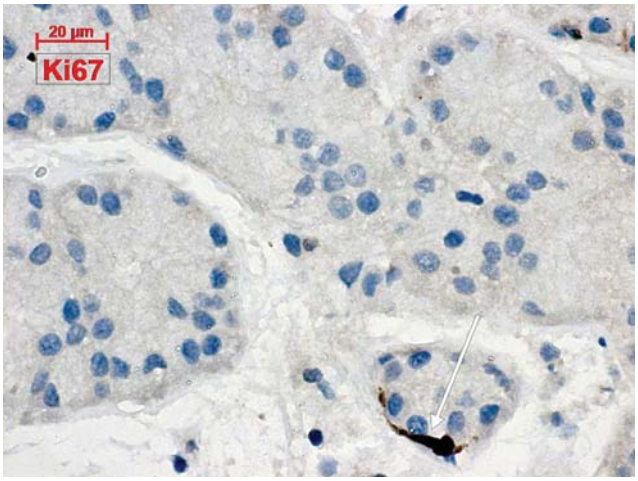


Figure 6. The proliferative marker Ki67 is scarcely expressed in an acinar cell resting on the basal lamina (arrow)

DISCUSSIONS

The peculiar morphology of MECs was assessed previously in the rabbit lacrimal gland by three-dimensional reconstructions which indicated that they have an octopus-like appearance with long cytoplasmic projections (Ding et al., 2005), such as we found here on bi-dimensional cuts.

Expression of podoplanin was previously detected in myoepithelial cells of salivary glands (Amano et al., 2011), as well as of lingual glands (Noda et al., 2010). It was suggested that the expression of podoplanin in the acinar epithelial cells and the MECs of the submandibular and sublingual salivary glands could be related to the mucous saliva excretion (Hata et al., 2008). However, although the orbit was checked for the expression of podoplanin to identify lymphatics (Nakao et al., 2012), few studies documented the expression of this lymphatic marker in MECs of the lacrimal gland (**Table 1**).

Table 1. Expression of several markers in myoepithelial cells. EGFR: Epidermal Growth Factor Receptor. L2E3: a monoclonal antibody directed against liver metallothionein. GFAP: Glial Fibrillary Acidic Protein

Markers	Tissue	Reference
podoplanin	salivary glands	(Hata et al., 2008, Tsuneki et al., 2013)
EGFR	mammary tissue	(Gama et al., 2009)
P-cadherin	mammary tissue	(Gama et al., 2004, Reis-Filho et al., 2003)
alpha-smooth muscle actin	mammary tissue, lacrimal gland	(Corben and Lerwill, 2009, Kivela, 1992)
calponin	mammary tissue	(Corben and Lerwill, 2009, Sanchez-Cespedes et al., 2013, Batistatou et al., 2003, Foschini et al., 2000)
smooth muscle myosin heavy chain	mammary tissue	(Corben and Lerwill, 2009, Batistatou et al., 2003)
cytokeratin 5	mammary tissue, lacrimal gland	(Kivela, 1992, Deugnier et al., 2002)
cytokeratin 13	lacrimal gland	(Kivela, 1992)
cytokeratin 14	mammary tissue, lacrimal gland	(Reis-Filho et al., 2003, Kivela, 1992, Sanchez-Cespedes et al., 2013)
CD10	mammary tissue	(Sanchez-Cespedes et al., 2013)
L2E3	salivary glands	(van den Oord et al., 1993)

Markers	Tissue	Reference
p63	mammary tissue	(Reis-Filho et al., 2003, Batistatou et al., 2003)
maspin	mammary tissue	(Reis-Filho et al., 2003, Deugnier et al., 2002)
S-100 protein	mammary tissue, lacrimal gland	(Reis-Filho et al., 2003, Kivela, 1992)
heavy caldesmon	mammary tissue	(Batistatou et al., 2003, Foschini et al., 2000)
CD109	mammary, salivary, and lacrimal glands	(Hasegawa et al., 2007)
vimentin	lacrimal gland	(Kivela, 1992)
GFAP	lacrimal gland	(Kivela, 1992)

In this study was assessed the scarce expression of Ki67 in basal cells of the glandular acini. However, the proliferative marker Ki67 was neither expressed in MECs, nor in acinar cells. On the other hand, scarce basal cells of the acini expressed the progenitor marker c-kit. This results support previous findings in murine lacrimal glands of nestin-, and Ki67-expressing proliferating cells which are a source of stem cells intermingled among acinar cells and MECs (You et al., 2011). It was suggested that such stem cells enter an epithelial-mesenchymal transformation to generate mesenchymal stem cells which, in turn, generate glandular epithelial cells via mesenchymal-epithelial transition (You et al., 2012). But MECs could also supply the epithelial niche, as it was demonstrated that MEC-derived progenitors of the surface epithelium progressively extinguish α-SMA expression while adopting a basal cell phenotype (Lynch et al., 2018). In these regards, the c-kit-expressing MECs we found in the lacrimal gland could just be cells which switched to a progenitor phenotype.

CONCLUSIONS

Podoplanin is expressed in MECs of the lacrimal gland, thus it should be added to the already known specific markers of these cells.

REFERENCES

1. Makarenkova HP, Dartt DA. Myoepithelial Cells: Their Origin and Function in Lacrimal Gland Morphogenesis, Homeostasis, and Repair. Curr Mol Biol Rep. 2015;1(3):115-23.
2. Nagato T, Yoshida H, Yoshida A, Uehara Y. A scanning electron microscope study of myoepithelial cells in exocrine glands. Cell Tissue Res. 1980;209(1):1-10.
3. Lemullois M, Rossignol B, Mauduit P. Immunolocalization of myoepithelial cells in isolated acini of rat exorbital lacrimal gland: cellular distribution of muscarinic receptors. Biol Cell. 1996;86(2-3):175-81.
4. Chaudhry AP, Schmutz JA, Cutler LS, Sunderraj M. Prenatal and postnatal histogenesis of myoepithelium in hamster submandibular gland. An ultrastructural study. J Submicrosc Cytol. 1983;15(3):787-98.
5. Ding C, Wong J, Mircheff AK, Schechter JEJTOS. Myoepithelial cells and neuronal nitric oxide synthase in the rabbit lacrimal gland. 2005(3):S57.
6. Amano I, Imaizumi Y, Kaji C, Kojima H, Sawa Y. Expression of podoplanin and classical cadherins in salivary gland epithelial cells of klotho-deficient mice. Acta Histochem Cytochem. 2011;44(6):267-76.
7. Noda Y, Amano I, Hata M, Kojima H, Sawa Y. Immunohistochemical examination on the distribution of cells expressed lymphatic endothelial marker podoplanin and LYVE-1 in the mouse tongue tissue. Acta Histochem Cytochem. 2010;43(2):61-8.

8. Hata M, Ueki T, Sato A, Kojima H, Sawa Y. Expression of podoplanin in the mouse salivary glands. Arch Oral Biol. 2008;53(9):835-41.
9. Nakao S, Hafezi-Moghadam A, Ishibashi T. Lymphatics and lymphangiogenesis in the eye. J Ophthalmol. 2012;2012:783163.
10. Tsuneki M, Maruyama S, Yamazaki M, Essa A, Abe T, Babkair HA, Ahsan MS, Cheng J, Saku T. Podoplanin is a novel myoepithelial cell marker in pleomorphic adenoma and other salivary gland tumors with myoepithelial differentiation. Virchows Arch. 2013;462(3):297-305.
11. Gama A, Gartner F, Alves A, Schmitt F. Immunohistochemical expression of Epidermal Growth Factor Receptor (EGFR) in canine mammary tissues. Res Vet Sci. 2009;87(3):432-7.
12. Gama A, Paredes J, Albergaria A, Gartner F, Schmitt F. P-cadherin expression in canine mammary tissues. J Comp Pathol. 2004;130(1):13-20.
13. Reis-Filho JS, Milanezi F, Paredes J, Silva P, Pereira EM, Maeda SA, de Carvalho LV, Schmitt FC. Novel and classic myoepithelial/stem cell markers in metaplastic carcinomas of the breast. Appl Immunohistochem Mol Morphol. 2003;11(1):1-8.
14. Corben AD, Lerwill MF. Use of Myoepithelial Cell Markers in the Differential Diagnosis of Benign, In situ, and Invasive Lesions of the Breast. Surg Pathol Clin. 2009;2(2):351-73.
15. Kivela T. Antigenic profile of the human lacrimal gland. J Histochem Cytochem. 1992;40(5):629-42.
16. Sanchez-Cespedes R, Suarez-Bonnet A, Millan Y, Guil-Luna S, Reymundo C, Herraez P, Espinosa de Los Monteros A, Martin de Las Mulas J. Use of CD10 as a marker of canine mammary myoepithelial cells. Vet J. 2013;195(2):192-9.
17. Batistatou A, Stefanou D, Arkoumani E, Agnantis NJ. The usefulness of p63 as a marker of breast myoepithelial cells. In Vivo. 2003;17(6):573-6.
18. Foschini MP, Scarpellini F, Gown AM, Eusebi V. Differential Expression of Myoepithelial Markers in Salivary, Sweat and Mammary Glands. Int J Surg Pathol. 2000;8(1):29-37.
19. Deugnier MA, Teuliere J, Faraldo MM, Thiery JP, Glukhova MA. The importance of being a myoepithelial cell. Breast Cancer Res. 2002;4(6):224-30.
20. van den Oord JJ, Sunardhi-Widyaputra S, Van Damme B, De Ley M. Monoclonal antibody to liver metallothionein: a novel marker for myoepithelial cells. Pathol Res Pract. 1993;189(10):1187-90.
21. Hasegawa M, Hagiwara S, Sato T, Jijiwa M, Murakumo Y, Maeda M, Moritani S, Ichihara S, Takahashi M. CD109, a new marker for myoepithelial cells of mammary, salivary, and lacrimal glands and prostate basal cells. Pathol Int. 2007;57(5):245-50.
22. You S, Kublin CL, Avidan O, Miyasaki D, Zoukhri D. Isolation and propagation of mesenchymal stem cells from the lacrimal gland. Invest Ophthalmol Vis Sci. 2011;52(5):2087-94.
23. You S, Avidan O, Tariq A, Ahluwalia I, Stark PC, Kublin CL, Zoukhri D. Role of epithelial-mesenchymal transition in repair of the lacrimal gland after experimentally induced injury. Invest Ophthalmol Vis Sci. 2012;53(1):126-35.
24. Lynch TJ, Anderson PJ, Rotti PG, Tyler SR, Crooke AK, Choi SH, Montoro DT, Silverman CL, Shahin W, Zhao R, Jensen-Cody CW, Adamcakova-Dodd A, Evans TIA, Xie W, Zhang Y, Mou H, Herring BP, Thorne PS, Rajagopal J, Yeaman C, Parekh KR, Engelhardt JF. Submucosal Gland Myoepithelial Cells Are Reserve Stem Cells That Can Regenerate Mouse Tracheal Epithelium. Cell Stem Cell. 2018;22(5):779