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Genetic Defect in Submucosal Gland Associated Caveolin-3: A New Paradigm in Esophageal Adenocarcinoma Risk

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Barrett's esophagus (BE) develops as a reparative metaplastic response to injury from gastroesophageal reflux disease (GERD) in the distal esophagus, and is a precursor to esophageal adenocarcinoma (EAC). Glandular origins of BE, either from gastroesophageal junction glands or esophageal submucosal glands (ESMG), have been proposed.[1] Similar to pancreatic cancer, acinar-ductal-metaplasia (ADM) transformation of ESMG has been associated with BE and EAC.[2] Here, we report the discovery of a deleterious germline mutation in ESMG-associated Caveolin 3 (*CAV3)*, segregating in a large family with BE

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and EAC, and propose a novel paradigm connecting ESMG-CAV3 in esophageal injury and disease susceptibility.

The NN-0001 pedigree (Figure 1A) was identified and recruited in institutional review board-approved studies at University Hospitals of Cleveland Medical Center and the Hospital of University of Pennsylvania. The proband was diagnosed with BE and high-grade dysplasia at age 52. The proband's brother had died of EAC at age 50 and mother had BE diagnosed at age 60 and breast cancer at age 68. Two maternal uncles were deceased from EAC at ages 67 and 72, respectively; a maternal male cousin, was diagnosed with BE at age 40 and underwent esophagectomy for a T1N0 EAC at age 57; and a maternal aunt, II-3, had BE diagnosed at age 46 and breast cancer at age 57.

Exomic sequencing identified a stop-gain (p.C19*) mutation in *CAV3* (GRCh38.p13, rs1182984115: chr3:8733933_C>A, minor allele frequency < 0.00001; see Methods) segregating in affected individuals (Figure 1A) This rare/private *CAV3* C19* null variant in *CAV3* was the only coding-variant that segregated in all but one of eight phenotyped family members. For this single family, assuming a dominant one-locus two allele model at zero recombination fraction, the LOD score for the putative causative *CAV3* null variant is 0.56 (p=0.023). Assessment of the C19* variant in mammalian cells showed loss of full-length CAV3 protein, underscoring the loss-of-function nature of the *CAV3* C19* variant (Figure 1B).

Caveolins are small integral membrane proteins that are the main component of caveolae, small raft like invaginations of the plasma membrane, implicated in a variety of cell functions including migration, differentiation, proliferation, and signal transduction.[3] *CAV3* is the predominant caveolar protein in striated and cardiac muscle. Missense variants of *CAV3* potentially affect oligomerization and scaffolding of *CAV3* and are associated with muscular dystrophies and cardiomyopathies.[3] However, family NN-0001 members with this loss-of-function *CAV3* C19* variant have no history of muscular dystrophy or cardiomyopathy, suggesting that this *CAV3* loss of function mutation is pleiotropic. We accordingly sought to explore the expression and putative function of CAV3 in the esophagus and its connection to BE/EAC.

Interrogation of CAV3 RNA and protein, using RNA sequencing and immunohistochemistry (IHC), respectively, showed lack of CAV3 expression in normal esophageal squamous (SQ), normal gastric, BE, and EAC tissues (Figure 1C). Given this absence of CAV3 from all these normal and diseased epithelia, we turned our attention to an affected individual from family NN-0001 who carried the C19* *CAV3* null variant. An esophagectomy specimen (Figure 1D) demonstrated ESMGs with ADM that showed striking atypia and a loss of myoepithelial cells. CAV3 was not present in the metaplastic acinar cells of these ESMG. Comparing this to wild-type CAV3 specimens focused our attention on a possible pathogenic role of ESMGs in the *CAV3* null variant. As expected, normal mucinous ESMGs from wild type CAV3 revealed mild to moderate CAV3 staining in myoepithelial cells (Figure 1E). However, the injured esophagectomy specimen from a presumed wild type individual showed light to moderate membranous and cytoplasmic CAV3 staining in metaplastic acinar cells of inflamed ESMGs; CAV3 was also expressed in proliferating ducts

arising from metaplastic ESMG and in the healing overlying neo-squamous epithelium. These findings are particularly striking given the known relationship between acinar ductal metaplasia (ADM) and BE/EAC.[2]

Incidentally, our search of ESMGs in archived esophagectomy specimens also found that CAV3 positive cells could be present at the base of multi-layered epithelium (Supplementary Figure) associated with ESMGs, a niche that is proposed to harbor BE precursor cells.[1]

Since murine models (such as mice and rats) lack ESMGs, we adopted a unique *in vivo* porcine injury model to orthogonally interrogate the potential role of caveolins in the porcine esophagus. In the normal/quiescent state, CAV3 protein was only present in myoepithelial cells surrounding acini in ESMGs and co-localized with alpha smooth muscle actin (aSMA), a known marker for myoepithelial cells. No CAV3 was present in interlobular ducts or in normal SQ epithelium (Figure 1F). Intriguingly, in our established porcine model,[4] 7 days following radio frequency ablation (RFA)-induced esophageal injury, expression of CAV3 was visible in metaplastic cells of dilated acini in ESMGs near the healing wound; CAV3 expressing cells were also evident in dilated interlobular ducts associated with ESMGs and throughout the healing neo-SQ epithelium adjacent to the wound (Figure 1F). The porcine injury model thus replicated the findings of the injured human esophagus.

Our study confirms the role of ESMGs in normal esophageal SQ healing after injury that has recently been demonstrated in human subjects undergoing RFA.[5] CAV3 is expressed in the metaplastic ESMG acini, dilated proliferating ducts, and the neo-squamous epithelium that repairs the injured esophagus. We postulate that loss of CAV3 function impacts normal esophageal homeostasis via disrupted ESMGs and impairs responses to injury-repair-healing, facilitating the development of BE (Figure 1G). These metaplastic acinar cells could arise from trans-differentiation of normal ESMG cells, trans-commitment of acinar precursor cells, or migration of precursor cells from the surrounding stroma. We suggest that one hypothesis to pursue is that after injury, CAV3 expressing cells may represent induced myoepithelial cells that migrate to heal the injured epithelium, similar to submucosal glands of the trachea where myoepithelial cells demonstrate the plasticity to migrate, differentiate and repair damaged epithelium.[6, 7] In summary, our study implicates ESMG-associated CAV3 expressing cells as critical players in esophageal homeostasis, revealing new paradigms on the origins of BE/EAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Que J, Garman KS, Souza RF, et al. Pathogenesis and Cells of Origin of Barrett's Esophagus. Gastroenterology 2019;157:349–364 e1. [PubMed: 31082367]
2. Garman KS, Kruger L, Thomas S, et al. Ductal metaplasia in oesophageal submucosal glands is associated with inflammation and oesophageal adenocarcinoma. Histopathology 2015;67:771–82. [PubMed: 25847432]
3. Parton RG, del Pozo MA. Caveolae as plasma membrane sensors, protectors and organizers. Nat Rev Mol Cell Biol 2013;14:98–112. [PubMed: 23340574]
4. Kruger L, Gonzalez LM, Pridgen TA, et al. Ductular and proliferative response of esophageal submucosal glands in a porcine model of esophageal injury and repair. Am J Physiol Gastrointest Liver Physiol 2017:ajpgi 00036 2017.
5. Konda V, Souza RF, Dunbar KB, et al. An Endoscopic and Histologic Study on Healing of Radiofrequency Ablation Wounds in Patients With Barrett's Esophagus. Am J Gastroenterol 2022;117:1583–1592. [PubMed: 35970814]
6. Tata A, Kobayashi Y, Chow RD, et al. Myoepithelial Cells of Submucosal Glands Can Function as Reserve Stem Cells to Regenerate Airways after Injury. Cell Stem Cell 2018;22:668–683 e6. [PubMed: 29656943]
7. Lynch TJ, Anderson PJ, Rotti PG, et al. Submucosal Gland Myoepithelial Cells Are Reserve Stem Cells That Can Regenerate Mouse Tracheal Epithelium. Cell Stem Cell 2018;22:653–667 e5. [PubMed: 29656941]

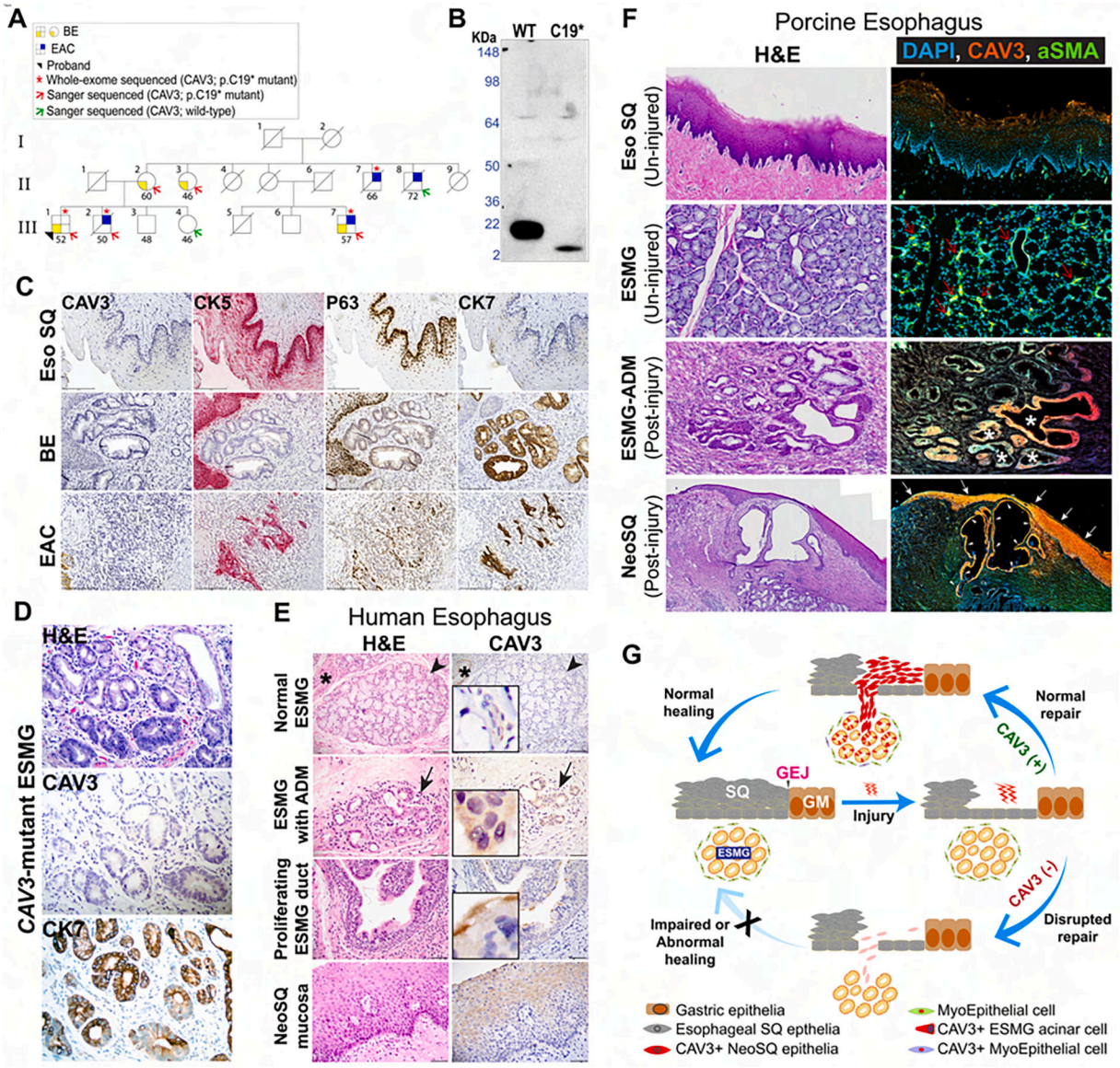

Figure 1:

Panel A: Pedigree NN-0001 - Yellow square in left lower quadrant = BE; Blue right upper quadrant = EAC. Proband is indicated with arrowhead. Age of diagnosis, affected individuals whose exomes were sequenced, and individuals carrying the null *CAV3* mutation are indicated. ***Panel B:*** Expression of C19* variant in mammalian cells shows greatly attenuated expression of small peptide fragment in left lane compared to wild type *CAV3* in left lane. ***Panel C:*** Immunohistochemistry (IHC) shows absence of CAV3 in normal human esophagus, BE, and EAC (left column, scale bar = 150 μm). Images in remaining columns demonstrate CK5, p63, and CK7 immunostaining in same tissues. ***Panel D:*** An ESMG found in an esophagectomy sample from the affected family member with *CAV3* mutant with BE and EAC. Histology (50 μm images) demonstrates an ESMG with ADM and atypia (top – H&E) with absence of myoepithelial cells; lack of CAV3 (middle - IHC); and strong patchy CK7 (bottom - IHC). ***Panel E:*** Paired H&E and CAV3 IHC images from

uninjured and injured esophagus. Top row - 100 μm image of normal mucinous ESMG from uninjured esophagus; IHC demonstrates CAV3 in myoepithelial cells (see magnified inset) around acini, asterisk indicates arterioles with CAV3 as positive controls. Next three rows are from injured esophagus. Second row - 50 μm image of ESMG with ADM in injured esophagus; note, arrows (H&E, left image and IHC, right image) indicate metaplastic acini surrounded by inflammatory infiltrate, magnified inset shows that CAV3 in metaplastic acinar cells appears both cytoplasmic and membranous. Third row - 50 μm images of proliferating inflamed duct associated with ADM, in magnified inset CAV3 staining appears more intense on luminal border. Bottom row – 50 μm images of post-injury neosquamous esophagus, CAV3 is seen in stratified neosquamous epithelium. ***Panel F:*** Porcine esophagus was evaluated to detect CAV3 and aSMA by immunofluorescence in uninjured esophagus. Representative regions within a 1000 μm composite image section are shown to demonstrate ESMGs, ducts, and squamous epithelium. Nuclear staining with DAPI is in blue, CAV3 is in red, and aSMA is in green. Top row – normal uninjured squamous epithelium did not demonstrate CAV3 or aSMA. Second row - in contrast, myoepithelial cells within quiescent ESMGs co-expressed CAV3 and aSMA in the uninjured pig esophagus; cells with yellow color indicated by red arrows demonstrate co-localization of CAV3 and aSMA. Third row - during esophageal wound healing, 7 days following radiofrequency ablative injury, white asterisks indicate CAV3 expressing metaplastic epithelial cells in dilated acini within ESMG under the wound; note inflammatory infiltrate in ESMG. Fourth row – white arrowheads indicate ESMG associated dilated proliferating ducts showing CAV3 expressing cells and again note inflammatory infiltrate surrounding ducts; white arrows indicate CAV3 expressing cells in neosquamous epithelium of the healing wound. Note difference between normal epithelium in top panel and inflamed healing epithelium in bottom panel. ***Panel G:*** Diagram representing hypothesis on how disruption of CAV3 leads to a susceptibility to BE. Our loss of function CAV3 variant disrupts normal esophageal homeostasis and leads to aberrant metaplastic repair after chronic injury. One hypothesis is that similar to airway and tracheal healing after injury,[6, 7] CAV3 expressing myoepithelial cells within ESMG may undergo epithelial transition and migrate to normally heal injured squamous esophagus.