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| **EM REPORT** |

**Electron Microscopy Laboratory**

**NIEHS-CMPB**

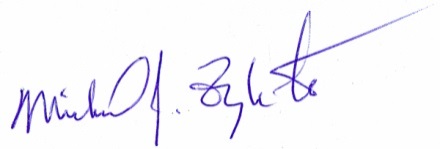
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**Signed** 

**date:**  **June 1, 2018**

**subject:** **Suen-Wallach: 455-C36: DIR-Uterine deletion of SIX1 disrupts epithelial basement membrane.**

The following samples were processed for TEM and examined ultrastructurally:

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| ***TEM #*** | ***Tissue*** | ***Sponsor ID*** | ***TEM Samples (Evaluated)*** |
| **45962-1A** | Uterine Horn | 1A-Uterine Horn | 0001-0015 |
| **45963-1B** | **Uterine Horn** | 1B-Uterine Horn | **0001-0008** |
| **45966-2A** | **Uterine Horn** | **2A-Uterine Horn** | **0001-0004** |

**Block Numbers for all samples processed 2/5/18:**

45962: 1A-Uterine Horn

45963: 1B-Uterine Horn

45964: 1C-Uterine Horn

45965: 1D-Uterine Horn

45966: 2A-Uterine Horn

45967: 2B-Uterine Horn

45968: 2C-Uterine Horn

45969: 2D Uterine Horn

**Materials and Methods**

The cells were fixed in 4F:1G. The cell pellets were embedded in 3% water agar following rinses in phosphate buffer as described in SOP 6.0.0. Subsequent processing of the cell samples was done according to SOP 6.0.0, using a Leica EM TP processor. Samples were rinsed with buffer, post-fixed in 1% osmium tetroxide in phosphate buffer, rinsed in water and dehydrated in an ethanolic series culminating in acetone. The samples were then infiltrated with Poly/Bed 812 epoxide resin. After polymerization, selected blocks were trimmed and semithin sections (approximately 0.5 µm thick) were cut, mounted on glass slides, and stained with 1% toluidine blue O in 1% sodium borate as per SOP 9.0 prior to being examined with a light microscope. After trimming the block faces down to areas of interest, ultrathin sections (80-90 nm thick) were cut from selected blocks, placed onto 200 mesh copper grids and then stained with uranyl acetate and lead citrate as per SOP 9.0. Digital images were captured with a Gatan Orius SC1000/SC600 attached to a FEICO Tecnai T12 transmission electron microscope.

**All of the images for this study can be found at : herbertemlab(**[**\\ntp-nas**](file:///\\ntp-nas)**)(Q:) in the folder named suen-wallach, subfolder Suen-Wallach 455-C36, TIFF folder.**

**Results and Discussion**

Three different samples of uterine horn from knockout mice were examined. The study focused on looking for abnormal peri-glandular cells that potentially arose from glandular epithelial cells.

**Sample 1A-Uterine Horn (Block 45962-1A)**

A low magnification image (45962-0001) shows the epithelial cells lining the lumen of a uterine gland. The cells have a few microvilli at the surface, a number of mitochondria, nuclei with variable shapes which often contain a single prominent nucleolus, and very little heterochromatin. Electron densities can be seen between portions of some of the cells on the luminal side of the gland that are junctional complexes. The basal side of the epithelial cells have thin basal laminae. One polymorphonuclear monocyte (PMN) can be seen in a capillary at the left center of the image. Image 45962-0010 shows epithelial cells with features as described above. Of note is the “peg” of cells with different morphology extending into the bottom of the epithelial cell layer from the lower left of the image. Also, there is a PMN in the upper left of the image. A higher magnification image of the epithelium (45962-0008) shows prominent rough endoplasmic reticulum profiles consisting of tubular structures studded on the outside with ribosomes. An even higher magnification view (45962-0011) of image 45962-0010 shows some of the “peg” cells. These cells are filled with proteinaceous microfilaments (see image 45962-0006), relatively sparse RER, and a few mitochondria. Collagen fibrils can be seen in the upper left and bottom center of image 45962-0006. There is no clearly defined basal lamina. Some electron densities are scattered amongst the microfilaments. If a clearly defined basal lamina was present, these cells would be consistent with smooth muscle cells and the densities could be interpreted as laminin. On the other hand, lacking a basal lamina, these might be modified epithelial cells containing cytokeratin microfilaments. Cytochemical or immunocytochemical techniques could help verify their identity.

Image 44962-0013 shows a relatively electron-dense population of cells with somewhat unusual morphology extending from the stromal region up to the epithelial cell population. A high magnification view of the unusual cells (45962-0015) show apparent microfilaments occupying a large portion of the cytoplasm, a few electron densities amoungst the microfilaments, a few nuclei, and sparse RER. No clear basal laminae can be identified with these cells. Numerous collagen fibrils are located in the spaces between the cells in this image. In contrast to the cells containing microfilaments, the slightly less electron-dense cells above and to the right in the image contain copious RER and polysomes and no clearly discernable microfilaments. They also lack basal laminae. These features suggest that these cells are fibroblasts.

**Sample 1B-Uterine Horn (Block 45963-1B)**

Image 45963-0001 is a low magnification view of the glandular epithelium, with the luminal space to the left of the image. The epithelial cells are columnar, with prominent vacuoles. The nuclei are generally oval, with minimal heterochromatin and prominent nucleoli. The stromal region below the gland contains many fibroblasts, often with elongate nuclei, as well as a lot of collagenous matrix. The lumen of the gland (images 45963-0002, 45963-0006)) contains cells with numerous “vacuoles” consistent with secondary lysosomes. One of the luminal cells (image 45963-0004) has a single round nucleus with two electron dense peripheral patches (heterochromatin?) and numerous small “vacuoles” containing small amounts of debris as well as one large body with electron dense deposits consistent with a secondary lysosome. RER profiles are numerous. These features suggest that the cell in the gland lumen are macrophages. A capillary with an endothelial nucleus and a possible platelet fragment can be seen in image 45963-0006. Image 45963-0003 shows glandular epithelial cells as described above, with the gland lumen at the bottom of the image. Some of the cells contain electron-dense bodies near the gland lumen that may be secretory granules. A small number of cells protruding into the gland lumen (45963-0005) have features similar to those described for epithelial cells cited above. The cell at the center left of the image appears to contain several secondary lysosomes with electron dense deposits. Electron dense junctional complexes can be seen between three of the epithelial cells on the luminal side of the cells.

**Sample 2A-Uterine Horn (Block 45966-2A)**

A low magnification view of a normal-appearing gland is shown in image 45966-0001. In this image, the glandular epithelial cells have large numbers of secretory granules at the apical end of the cells and lesser numbers at the basal region. The lumen of the gland contains floculent material, and microvilli from the epithelial cells extend a short distance into the lumen. Image 45966-0002 is a higher magnification view of the columnar epithelial cells of the gland showing the features previously described. A thin basal lamina can be seen around the basal periphery of the gland. Image 45966-0004 is a high magnification view of the base of a glandular epithelial cell at the lower left of the image, showing the basal lamina clearly. The stromal region to the upper right of the image shows numerous collagen fibrils and two elongate portions of cells, consistent with fibroblasts.

**Overall Conclusions:**

Some of the glands had small populations of unusual-appearing cells associated with the basal surface of the glands. These cells did not have clear basal laminae, they had somewhat more electron-dense cytoplasm, sparse RER, and they contained large quantities of cytoplasmic microfilaments. Electron-dense deposits were associated with the microfilaments. If the cells had had clear basal laminae present, they would have been consistent with smooth muscle cells (SMCs), though this cell type is usually found toward the base of the stromal areas, rather than near the glands and the uterine epithelium. If these microfilaments could be identified immunocytochemically as cytokeratin microfilaments, this would suggest an epithelial origin for these cells. Without such evidence, their identity is uncertain.