Morphological changes to the vulvar squamous carcinoma cell line, A431, resulting from clobetasol-induced upregulation of vimentin

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INTRODUCTION

Lichen Sclerosus (LS) is an inflammatory skin condition that occurs on the genital area and mostly affects women ages 40-60 years old. Prolonged inflammation from LS has the potential to develop into vulvar cancer. Clobetasol is an anti-inflammatory glucocorticoid topical cream often used to treat the symptoms of LS but in vulvar epithelial cancer cell line A431, treatment with clobetasol causes changes in gene expression. Clobetasol treated cells, referred to as A431D, lose expression of E-cadherin, a tumor suppressor protein, in conjunction with the upregulation of vimentin, an intermediate filament protein. These changes in expression of E-cadherin and vimentin are often idicative of an epithelial-mesenchymal transition (EMT) and marks a more aggressive phenotype in cancer progression.. The loss of cytokeratins 8/18 is also associated with the EMT. However, A431 cells exposed to clobetasol retain the expression of these cytokeratins. The gene expression changes induced by clobetasol result in structural alteration of the cytoskeleton and loss of epithelial characteristics. Confocal microscopy was used to identify colocalization of vimentin and cytokeratin 8 in the A431D cells. We are also exploring the use of Raman spectroscopy (RS) to noninvasively identify the structural fingerprints of cytokeratins 8/18 and vimentin. This technique may help to observe the cellular reconstruction of treated A431 cells.

METHODOLOGY

Cell culture:
A431D cells were generated as described in Lewis et al., (1994). Cells were grown in DMEM, 10% FBS without clobetasol (A431) or with 10^{-7M} clobetasol (A431D). NIH3T3 (gift of Dr. James Wahl, Univ. of Nebraska Dental school) were grown in MEM, 10% FBS.

Antibodies:
The following antibodies were used for identifying cytokeratin 8, vimentin, glucocorticoid receptor and actin in Western blots and immunofluorescence and confocal microscopy: Mouse-anti-glucocorticoid antibody (BS13385R, BIOSS), Rabbit anti-vimentin (AMF-17bs, Developmental Studies Hybridoma Bank) and anti cytokeratin 8 (Millipore Sigma).

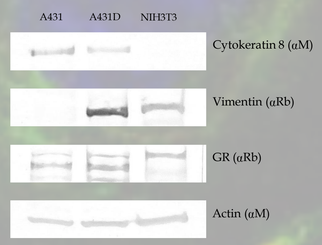
Western Blot:
Cells were extracted in Tris-acetate, EDTA buffer with Triton X-100 and protein was measured using a standard biorad reagent. Equal amounts of protein were separated on a 7% SDS-Gel and transferred to nitrocellulose and then probed using indicated antibodies followed by alkaline phosphate secondary.

Immunofluorescence and Confocal Microscopy:
All cells were grown on poly-L-lysine glass coverslips and plated at 1 x 10^5 cells/mL. Cells were fixed with HistoChoice (company name), blocked in 10% goat serum and incubated with primary antibody followed by appropriate secondary antibodies. Immunofluorescence microscopy images were taken using a Zeiss Axiophot microscope. Confocal images were taken using a Leica Stellaris DMI/8 microscope and processed with Imaris Viewer 10.1. 3D images had a resolution of 140 nm in the x and y directions and 240 nm in the z direction.

Raman Imaging:
Cells were grown as described above, processed using Histochoice, followed by incubation with Au 80 nm and mounted in a 1x borate buffer. Gold nanoparticle-coated cells were imaged using a Model Alpha 300, WITech - Oxford Instrument.

RESULTS

Figure 1. Cytokeratin 8 expression is maintained in A431D cells despite upregulation of vimentin caused by treatment with clobetasol. The relative expression of cytokeratin 8, vimentin, and glucocorticoid receptor (GR) in A431, A431D, and NIH3T3 was quantified by Western blot. A431 expresses cytokeratin 8 but not vimentin. A431D gains expression of vimentin while retaining expression of cytokeratin 8. NIH3T3 lacks expression of cytokeratin 8 and was used as a control for vimentin staining. The relative expression of GR may be higher in A431D.



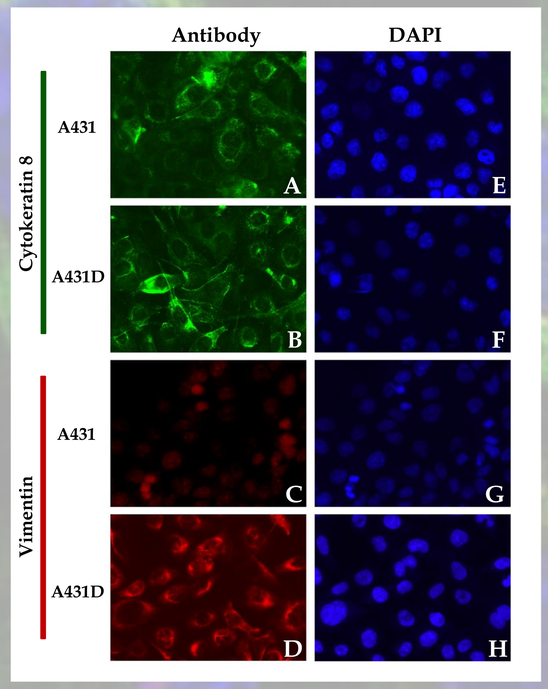


Figure 2. A431D cells display filamentous cytokeratin 8 similar to A431 cells and in addition to vimentin expression. Cytokeratin 8 (A & B) and vimentin (C & D) were imaged through indirect single immunofluorescence. DAPI (E, F, G, H) is bound to DNA in the nucleus as a counterstain. A431 and A431D express cytokeratin 8 (A & B). A431D gained the expression of vimentin whereas A431 did not (C & D) as a result of clobetasol treatment. The expression of vimentin continued in the A431D cells even when the clobetasol was removed. Vimentin-labeled A431 showed some background staining that appeared to be an artifact since it was localized to the nucleus (C & G). These images have been digitally colorized.

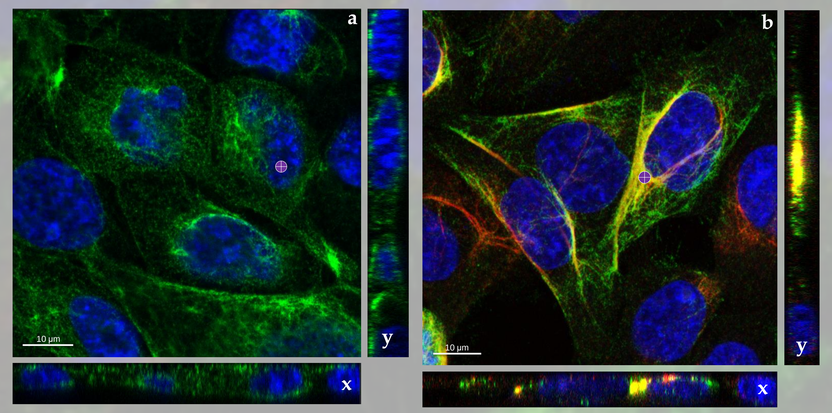


Figure 3. A431D cells expressing both vimentin and cytokeratin 8 show areas of colocalization. 3D images of immunofluorescent A431 (a), A431D (b), and NIH3T3 (c) cells. The x and y cross sections taken at the ⊕ indicator can be seen to the right and below the images. Cytokeratin 8, green, is found in the A431 and A431D samples. Vimentin, red, is found in A431D. A431D's colocalization of cytokeratin 8 and vimentin can be seen in yellow. NIH3T3 cells were used as a control for vimentin staining and localization.

DISCUSSION

The co-expression of cytokeratins 8/18 and vimentin is observed. This is unusual for cells that undergo an EMT. The potential interactions between the intermediate filament networks have yet to be confirmed. Gold nanoparticles may be able to increase the resolution of colocalized cytokeratins 8/18 and vimentin while revealing chemical information about their interaction.

FUTURE DIRECTIONS

Higher resolution of these changes in protein expression was attempted to be visualized by coating the cells in gold nanoparticles (80 nm). We have successfully captured SERS (Surface Enhanced Raman Scattering) signals. All major five spectral components (A-E) were extracted and the spectral assignments are in progress.

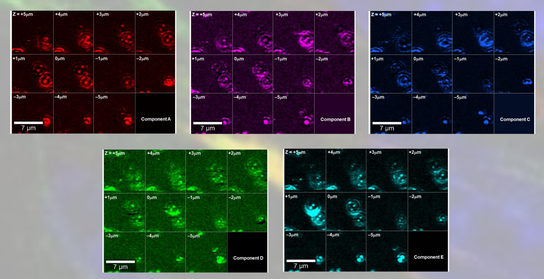


Figure 4. SERS imaging (10 μm × 10 μm) of each spectral component (A-E). This is a white-light image of A431 cells at longitudinal location (z) ranging from +5 μm to –5 μm.

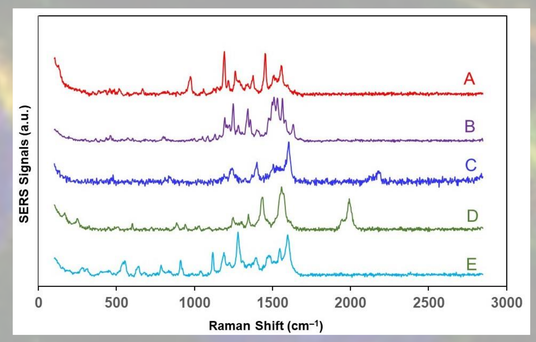


Figure 5. Unassigned SERS spectrum of each component (A-E). These spectra reveal chemical information about the nanoparticle-coated structure.

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REFERENCES

Clancy, A., Spaans, N., and Weberpals, I. The forgotten woman's cancer: vulvar squamous cell carcinoma (VSCC) and a targeted approach to therapy. Annals of Oncology. 2016;27(9):1696–1705.

Lewis, J. E., Wahl, J. K., Sass, K. M., Jensen, P. J., Johnson, K. R., & Wheelock, M. J. (1997). Cross-Talk between Adherens Junctions and Desmosomes Depends on Plakoglobin. The Journal of Cell Biology, 136(4), 919–934. doi:10.1083/jcb.136.4.919