Visualizing Altered Gene Expression of Clobetasol Treated Vulvar Carcinoma Cells with Raman Spectroscopy

Kia Haering
Dr. Kazushige Yokoyama, Department of Chemistry, and Dr. Jani Lewis, Department of Biology, SUNY Geneseo, Geneseo, NY, USA, 14454
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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, so management and treatment of symptoms is crucial. Clobetasol is an anti-inflammatory glucocorticoid topical cream often used to treat the symptoms of LS. In vulvar epithelial cancer cell line A431, treatment with clobetasol causes undesirable changes in gene expression. Treated cells undergo the loss of E-cadherin, a tumor suppressor protein, in conjunction with the upregulation of vimentin, an intermediate filament protein. These specific changes in expression of E-cadherin and vimentin are markers for the progression of the epithelial-mesenchymal transition (EMT). This transition allows cancer cells to become more aggressive and invasive. The loss of cytokeratins 8/18 is also associated with the EMT. A431 cells exposed to clobetasol partially 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. Raman spectroscopy (RS) can noninvasively identify the structural fingerprints of E-cadherin, cytokeratins 8/18, and vimentin. Raman Imaging can be used to observe the cellular reconstruction of treated A431 cells that undergo this transition towards a mesenchymal presenting structure.

METHODOLOGY

A431 and A431D (clobetasol treated) human vulvar cells were examined for the presence of E-cadherin, cytokeratins 8/18, and vimentin using indirect immunofluorescence. Cells were grown on glass coverslips in DMEM + 10% fetal bovine serum (FBS). After 2 days, cells were fixed with Histochoice followed by incubation with primary antibody, then with goat-anti-mouse conjugated secondary antibody. Cells were imaged using a Zeiss Axiophot immunofluorescence microscope. For Raman Spectroscopy prepared slides, the A431 cells were grown on poly-L-lysine treated coverslips in DMEM + 10% FBS. The A431 + clob (progressing cells) and A431D (progressed) cells were grown on poly-L-lysine treated coverslips in DMEM + 10% FBS + 10^{-7} M clobetasol. After 2 days, the cells were rinsed in 1x PBS and fixed in Histochoice for 10 minutes. The slides were rinsed in 1x PBS again then mounted with 1x PBS.

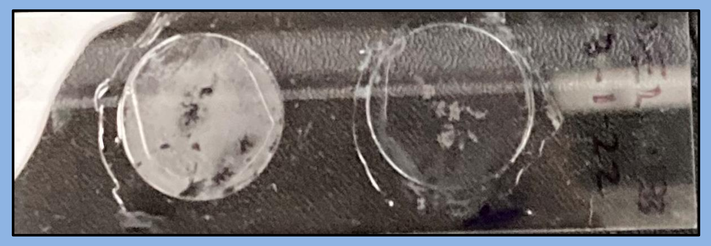

Figure 1. A431 & A431D cells mounted on glass coverslips for imaging using Raman Spectroscopy.

RESULTS

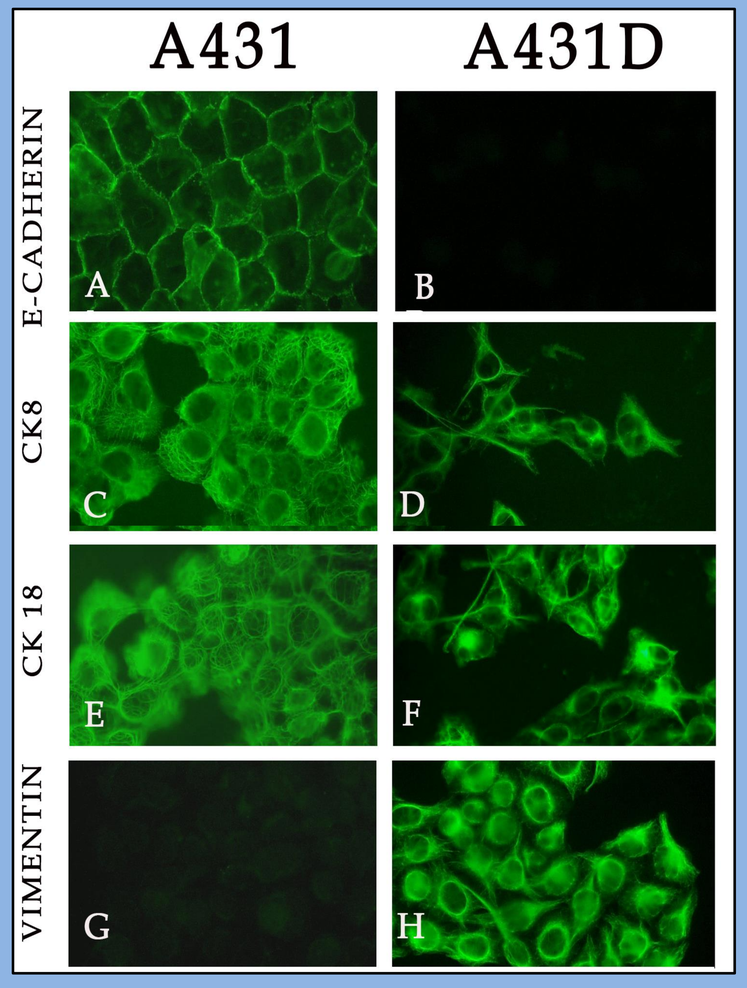

Figure 2. E-cadherin (A & B), cytokeratin 8 (CK 8; C & D), cytokeratin 18 (CK 18; E & F), and vimentin (G & H) were imaged through indirect immunofluorescence. E-cadherin staining is at cell-cell borders (A). A431 cells display a cobblestone-like appearance typical of epithelial cells and express cytokeratins 8 and 18 but not vimentin (A, C, E, & G). A431D cells lose E-cadherin expression but retain expression of cytokeratins 8 and 18 as well as expressing vimentin (B, D, F, & H). These cells take on a more elongated fibroblast-like appearance (D, F, & H).

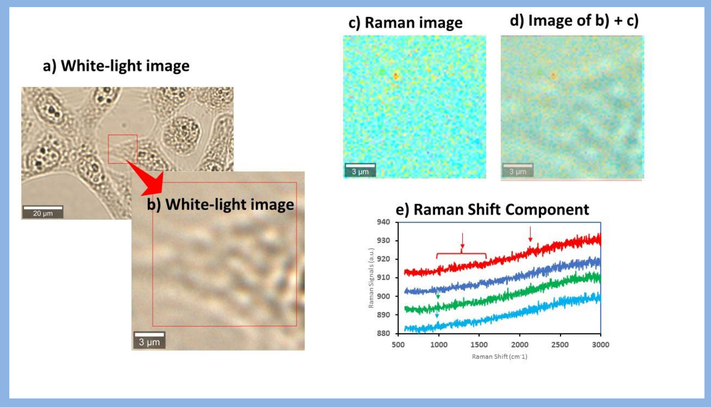

Figure 3. The Raman Imaging of A431 cells. (**a**) The Raman white-light image is shown. (**b**) The white-light image is enhanced. (**c**) The signal is weak in the Raman image and no specific cellular components are visible. (**d**) The white-light image and Raman image are overlayed. There are no distinct or discernable signals from the scanned location. (**e**) The scanned Raman spectrum is separated into components. The peaks indicate the presence of unspecific proteins and amino acids.

DISCUSSION

The current methodology was unsuccessful to produce distinct Raman signals of the surface proteins. Identifying the spectra of the proteins E-cadherin, cytokeratins 8/18, and vimentin is the first step in determining the change in cellular structure and molecular interactions.

FUTURE DIRECTIONS

Higher resolution of these changes in protein expression may be visualized by coating the cells in Au (80 nm) nanoparticles. This stabilizes the cells and provides a homogeneous surface for surface enhanced Raman scattering (SERS). Resolution of the changes may also be increased with a water immersion objective lens.

ACKNOWLEDGEMENTS

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REFERENCES

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