Identifying Changes in Protein Expression in Human Vulvar Carcinoma after exposure to Clobetasol using Raman Spectroscopy

Jennie Dworkin, Julia Malinchak, and Kia Haering
Dr. Kazushige Yokoyama, Department of Chemistry, and Dr. Jani Lewis, Department of Biology, SUNY Geneseo, Geneseo, NY, USA, 14454
Funded by the TRAC Grant

INTRODUCTION

E-cadherin is considered a tumor suppressor gene whose loss has been correlated with carcinogenesis. E-cadherin is expressed in squamous cells such as those from the vulvar epithelium. Cancers that have lost E-cadherin expression can indicate progression to a more serious state. A second marker of cancer progression in epithelial cells is expression of the intermediate filament, vimentin. Loss of E-cadherin and gain of vimentin expression are often used as markers in characterizing tumor biopsies. We have found that the vulvar cancer cell line, A431, when treated with clobetasol, down regulates E-cadherin and upregulates vimentin. These cells are referred to as A431D. Interestingly, these cells continue to express the epithelial cytokeratins 8 and 18. In trying to understand the influence of vimentin on the structure of these cells, we are using Raman spectroscopy (RS) to examine any interactions between cytokeratins 8 and 18 with vimentin. RS is a label-free and nondestructive spectroscopic method that has the potential to demonstrate efficacy in diagnostics and therapeutic response monitoring. Raman spectroscopy has been used to identify cell state transitions, including epithelial to mesenchymal transition (EMT) in cancer cells.

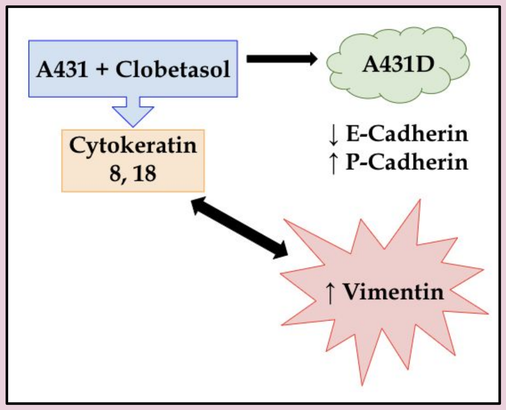

Figure 1. Schematic diagram of A431 & A431D cells and their corresponding capabilities.

METHODOLOGY

A431 and A431D human vulvar cells were examined for the presence of E-cadherin cytokeratin 8 and cytokeratin 18 and vimentin using indirect immunofluorescence. Cells were grown on glass coverslips in DMEM + 10% fetal bovine serum. After 2 days, cells were fixed with histochoice followed by incubation with primary antibody (cytokeratin 8 or 18 or vimentin or E-cadherin) followed by goat-anti-mouse conjugated secondary antibody. Cells were imaged using a Zeiss Axiophot immunofluorescence microscope.

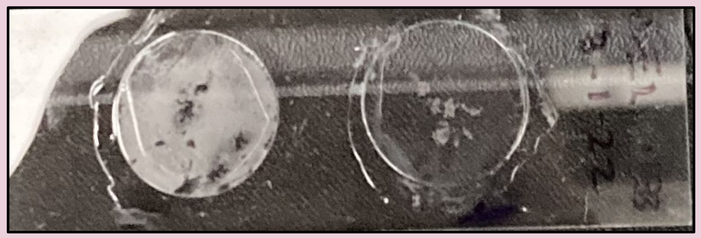

Figure 2. A431 & A431D cells mounted on glass coverslips for imaging using Raman Spectroscopy.

RESULTS

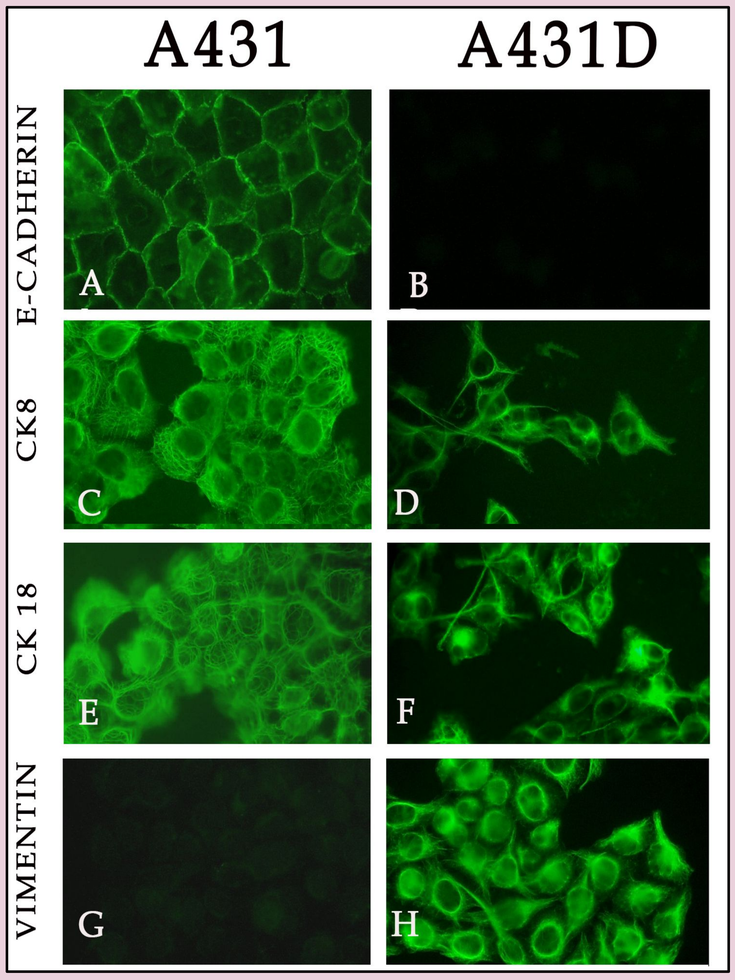

Figure 3. E-cadherin (A & B), cytokeratin 8 (CK 8; C & D), cytokeratin 18 (CK 18; E & F), and vimentin (G & H) were imaged through indirect immunofluorescence. E-cadherin staining is at cell-cell borders (A). A431 cells display a cobblestone-like appearance typical of epithelial cells and express cytokeratins 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).

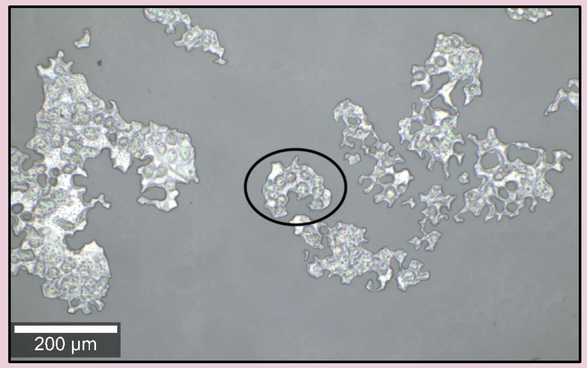

Figure 4. Raman imaging of A431 cells at 200 µm. The power of the Raman spectroscopy used to visualize the cells is 2.12 x 10^6 W/cm².

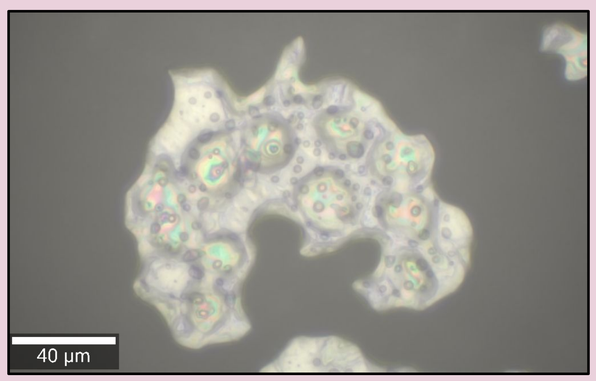

Figure 4a. Raman imaging of A431 cells at 40 µm. The power of the Raman spectroscopy used to visualize the cells is 2.12 x 10^6 W/cm².

DISCUSSION

Raman spectroscopy should be useful in discerning if vimentin impacts the organization of cytokeratin 8 and 18 by direct association or if these filaments do not interact and therefore do not directly influence one another. This can be done by identifying the spectra of cytokeratin 8 and 18 and vimentin in the cells, and using it to "digest out" their molecular interactions.

REFERENCES

J. Lewis. Identification and Classification of Changes in Protein Expression and Localization Associated with Epithelial to Mesenchymal Transitions in Human Vulvar Carcinomas after Exposure to the Glucocorticoid Analog, Clobetasol. *NSF-MRI* **2021**.

S.P.. Singh, et. al., Identification of morphological and biochemical changes in Keratin- 8/18 knock-down cells using Raman Spectroscopy. *Journal of Biophotonics* **2017**, *vol 1-8*.