The Quandary of Cellular Fractionation — Optimizing Ambion™ Paris System to Advance HPV16 Cancer Research

Emma Bisch[1], Caylin Billingsley[1,2], Rachel A. Katzenellenbogen[1,2,3]

Indiana University School of Medicine[1]; Indiana University School of Medicine Department of Microbiology and Immunology[2]; Indiana University School of Medicine Department of Pediatrics[3]

Background and Hypothesis: Human Papillomavirus (HPV) is the causative agent in nearly all cervical cancer cases. It has been shown that the HPV type 16 E6 protein interacts directly with the host protein NFX1-123. Short-term studies have shown that NFX1-123 remains in the cytoplasm; however, it has not been investigated whether NFX1-123 actually translocates to the nucleus in the long term. We hypothesize that over time, NFX1-123 translocates to the nucleus of the cell in long term cultures with 16E6. This present study seeks to optimize the Ambion™ Paris system to allow for pure, proper separation of the cytoplasmic and nuclear cellular compartments.

Project Methods: Three biologically unique backgrounds of human foreskin keratinocytes (HFKs) were cultured in a monolayer tissue culture dish. Using the Ambion™ Paris system, proteins were isolated as whole cell extracts, or nuclear and cytoplasmic fractions. For one lysis method, only protease inhibitors were added to the lysis buffers of the Ambion™ Paris system. For another lysis method, 1% NP-40 and protease inhibitors were added to the lysis buffers of the Paris system. Protein lysate concentrations were quantified, then purity of subcellular lysates was determined by western blotting. Histone H3 and GAPDH were used to identify nuclear and cytoplasmic compartments, respectfully.

Results: Western blotting confirmed that adding 1% NP-40 to the lysis reagents of the Ambion™ Paris kit optimized subcellular fractionization.

Potential Impact: Being able to efficiently separate the cytoplasmic and nuclear compartments will allow for accurate identification of NFX1-123 localization during long-term HPV16 infection. If NFX1-123 is found to move into the nucleus under the influence of HPV 16E6, then this could indicate potential transcriptional regulatory functions of the NFX1-123 protein during HPV infection, which is unique from its function in non-infected cells.