Deducing Function through Localization: PCV1 VP3 the Problem Child

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Abstract

The Circoviridae family of viruses have several members that encode proteins observed to induce apoptosis in human cells with specificity towards cancer cells. One unique example, PCV1-VP3, has significant morphological and functional differences, possessing an additional tail domain and localizing in the cytoplasm rather than the nucleus of transformed cells. To gain insight into possible mechanisms of action, compartmentalization of PCV1-VP3 was investigated using differential detergent fractionation and fluorescent microscopy.

Introduction

Due to the various mutations cancerous cells can accrue as they proliferate, treatments that act through novel pathways are valuable targets for research. One such potential group of treatments are the apoptotic proteins encoded in the genomes of the Circoviridae family of viruses. These proteins act with specificity towards cancerous cells. Apoptin, a protein found in Chicken Anemia Virus, is one well-characterized example. A particular circovirus protein, viral protein 3 of Porcine Circovirus Type 1 (PCV1 VP3), is unique. It is twice as large as these homologues, and possesses a C-terminal "tail" region in addition to the structurally homologous "core" domain. Despite the lack of homology, the tail domain exhibits powerful killing ability when separated from the core, which counter-intuitively loses its apoptotic properties when separated from the tail. Investigation into the cytoplasmic localization of PCV1-VP3 may provide insight into its mechanism of action.

Detergent Fractionation

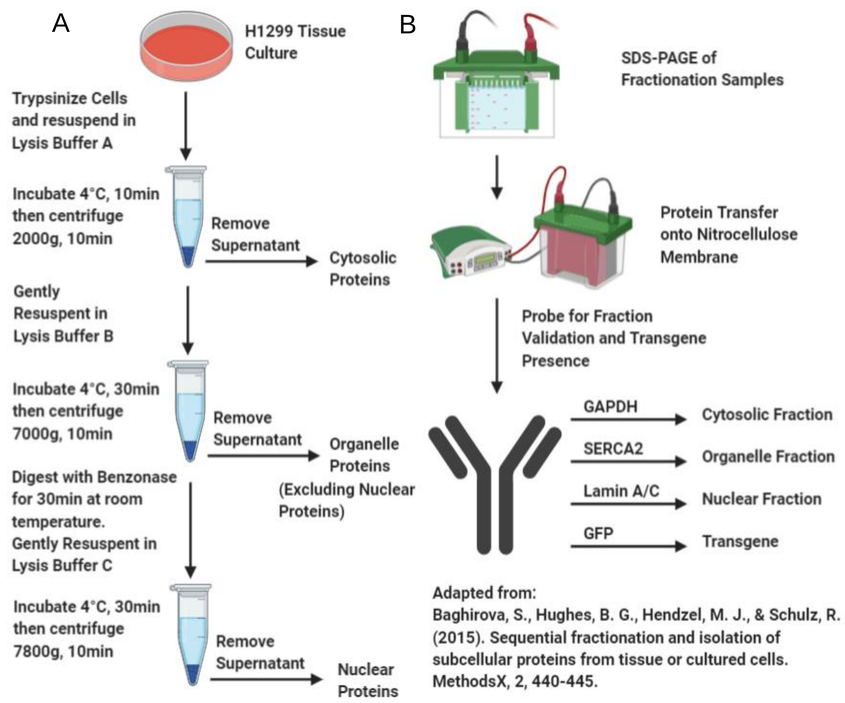

Figure 4. Differential Detergent Fractionation of Transfected Cells. (A). Transfected Cells are sequentially treated with different detergent cocktails designed to dissolve their corresponding membranes. **(B).** The resulting fractions are assessed via SDS-PAGE and subsequent Western Blotting against various cellular proteins to validate fraction identity and EGFP to determine the localization of PCV1 VP3.

ER Colocalization Studies

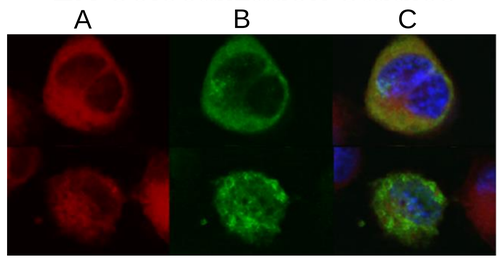

Figure 5. PCV1 VP3 Microscopy. Diffuse and punctate expression patterns under **(A)** ER stain and **(B)** GFP excitation, along with **(C)** a composite including DAPI nuclear stain. The diffuse cell (first row) exhibited noticeable yellow overlap, while the punctate cell (second row) showed little to no overlap.

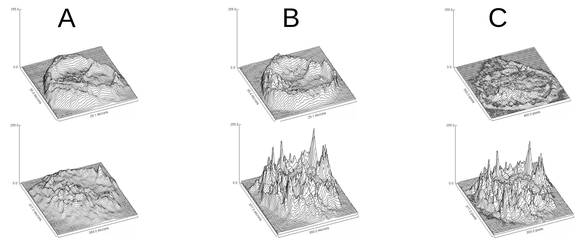

Figure 6. Surface Plots. Visualization of **(A)** ER stain intensity and **(B)** GFP intensity measured PCV1 VP3 microscopy in a diffuse and punctate expresser. Appearance of overlap in the diffuse cell is highlighted by **(C)** subtracting the GFP fluorescence intensity from ER stain fluorescence, as indicated by the significantly more flat appearance of the result. Subtracting ER from GFP intensity in the punctate cell illustrates significantly less colocalization.

Tetracycline Controlled Plasmid

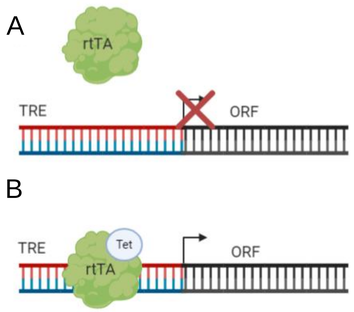

Figure 1. Tet-On System for Gene Regulation. In the Tet-On system, the rtTA must bind the TRE for transcription to occur. **(A)** The rtTA is not capable of this on its own, **(B)** but only in the presence of tetracycline (Tet) may it undergo a conformational change which allows it to do so.

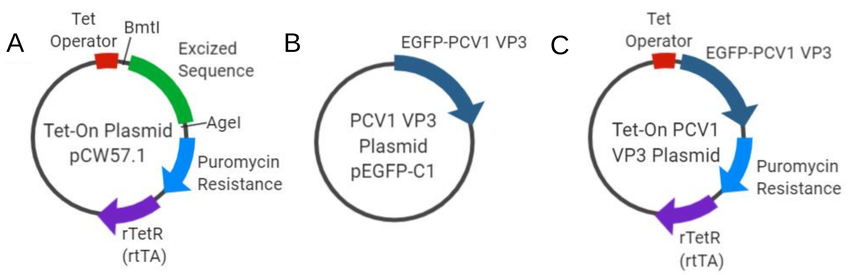

Figure 2. PCV1 VP3 Inducible Plasmid. (A). A Tet-On plasmid system containing both the TRE (Tet Operator) and the rtTA is restricted with BmtI and AgeI to remove the region directly downstream the TRE. **(B).** PCV1 VP3 conjugated to EGFP on its N-terminus is then PCRed out of its current plasmid utilizing custom primers designed to append BmtI and AgeI restriction sites to the 5' and 3' ends of the coding strand respectively. **(C).** The resulting PCR product is then restricted with BmtI and AgeI and ligated into the Tet-On plasmid.

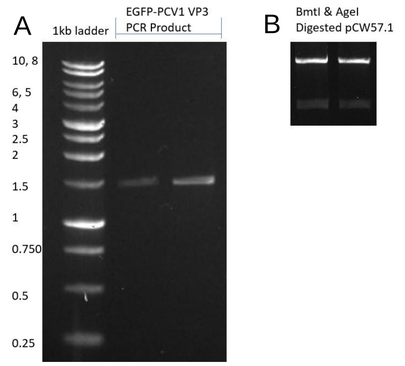

Figure 3. Molecular Cloning Products. (A). The resulting EGFP-PCV1 VP3 PCR product ran at ~1.5kb, just above its expected size of 1.4kb indicating successful PCR with the custom primers. **(B).** The restricted Tet-On plasmid resulted in two bands corresponding to the vector backbone and the excised sequence, validating construct identity.

Discussion

Completion and implementation of a tet-controlled plasmid would allow for DNA normalization and high expression facilitating subsequent experiments such as differential fractionations and DNA microarrays. Apoptosis specific microarrays in particular could probe against the expression of characterized death genes and help determine which pathways are being activated in transformed cells. Detergent fractionation results would alternatively indicate the broad localization of PCV1 VP3: cytoplasm, membrane-bound organelle, or the nucleus. Such results could provide independent confirmation to microscopy studies and other fractionation methods determining subcellular localization. Fluorescent microscopy did not disprove colocalization of PCV1 VP3 and the endoplasmic reticulum. However, these results alone are not conclusive, as the large size of the nucleus increases the likelihood that any two signals in the cytoplasm will appear to overlap. Significant noise in the images captured also decreased the power of quantitative statistical tests, making further investigation with higher quality samples necessary for achieving conclusive results.