MOL231: Investigating the behaviour of Vault RNPs in proliferating vs senescent cells.

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Background:

Vaults are large, hollow structures found in the cytoplasm of most eukaryotic cell types, consisting of both protein and RNA. Despite their high conservation from slime molds to humans (2), we still know surprisingly little about their function.

- Outer shell composed of 78 subunits of major vault protein (MVP) (1).
- Inside are the minor vault proteins TEP-1 and VPARP.
- At the cap one or more vRNAs may bind (vRNA1-1, 1-2, 1-3, 2-1).

Aim:

Grellscheid lab has recently found that Vault component levels increase in senescent U2OS cells (via RNA-seq and Mass spec). U2OS is a cancer cell line, and it is therefore of interest to determine whether this increase is a cancer-specific phenomenon, or whether it aslo occurs in primary cell lines.

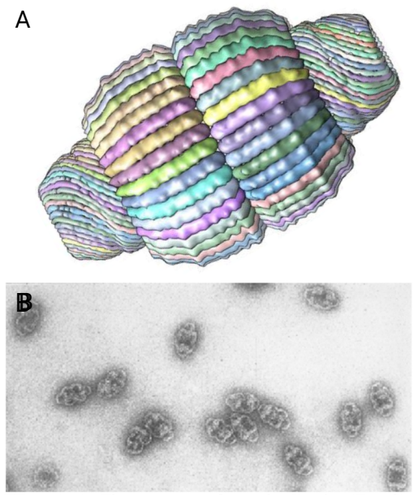

Figure 1. A - The structure of a vault complex (Tanaka et al, 2009). B - Electron micrograph of vaults in rat hepatocytes (Kedersha and Rome, 1986).

Method 1: Etoposide-induced senecence in primary-like cell lines (MRC5, AD2, RPE1).

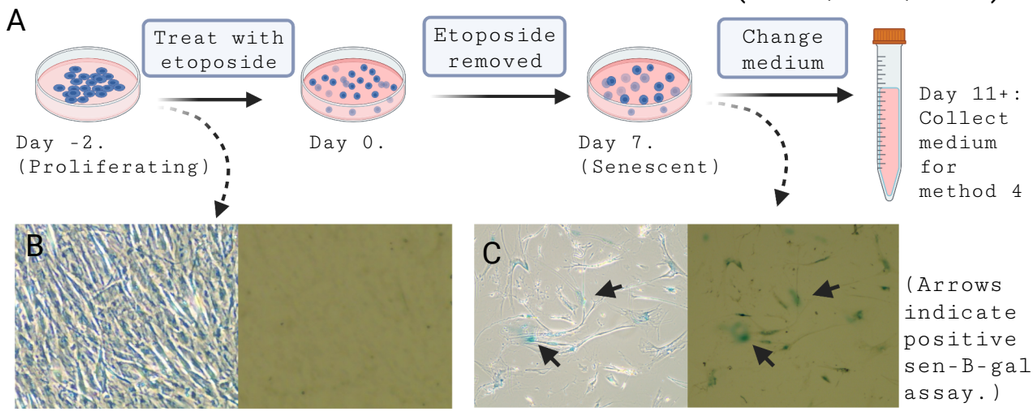

Figure 2: A) Diagram of protocol to induce senescence. (B and C) A sen-B-galactosidase assay was used to confirm when cell were senescent.

Method 2: Immunofluoresence microscopy to detect localisation of MVP in proliferating vs senescent cells.

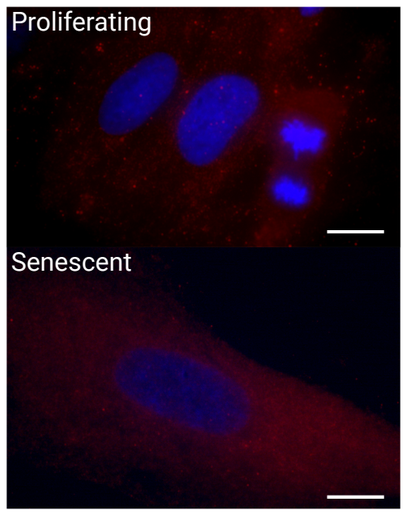

Figure 3. AD2 cells were incubated with anti-MVP (red) and DAPI (blue, nucleus). MVP is mainly located in the cytoplasm in both proliferating and senecent cells.

Method 3: qPCR primer design and validation for vault components.

RNA isolated from cells by Trizol extraction, then revesre transcribed to cDNA.
Primers were devolped and tested as shown in Figure 5. All primer pairs gave PCR products of expected lengths. The primers are now ready to be used for qPCR in the future to determine the expression levels of vault components in young vs senescent cells.

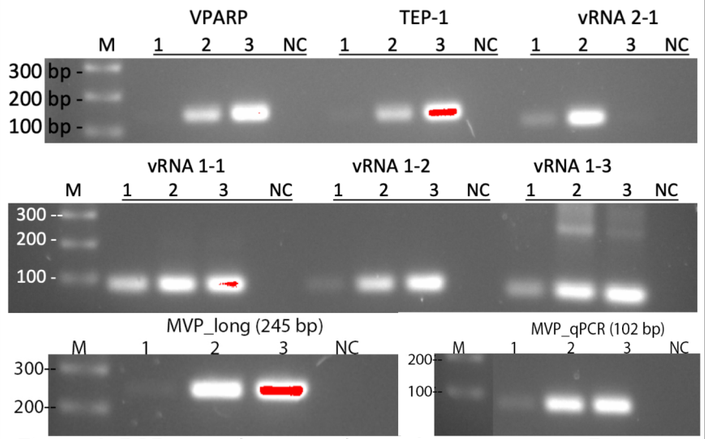

Figure 4. PCR test of primers for vault components. Primer pair indicated above wells. For all primers: 1- Poor quality, discarded, 2- RPE senescent, 3- U2OS senescent, NC-negative control.

Method 4: Ultracentrifugation of cell medium, and subsequent western blot to check whether vaults might be accumulating extracellularly.

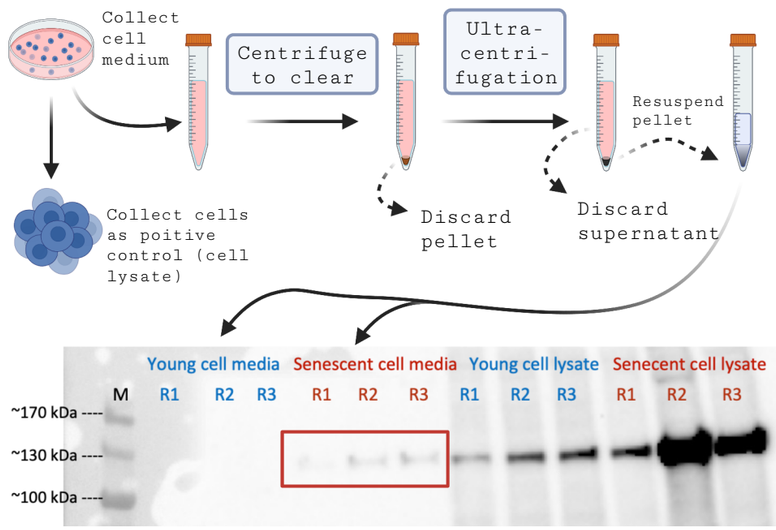

Figure 5. Western blot from AD2 cells, treated with anti-MVP. Red box indicates positive result for MVP in senescent cell medium, which is absent from young cell medium.

Further research:

- Use the primers validated in method 3 for qPCR of various cell lines to determine whether vault expression is increasing in senesence.
- Determine whether vault presence in cell medium is related to senescence, whether it is cell-type specific, and whether it is there because cells are secreteing them or whether stressed cells are bursting and releasing them.
- Investigate the effect of vaults in cell medium on neighboring cells.

References:

1) Kato K, Zhou Y, Tanaka H, Yao M, Yamashita E, Yoshimura M, et al. The structure of rat liver vault at 3.5 angstrom resolution 2009. doi:10.2210/pdb2zuo/pdb.

2) Kedersha NL, Miquel MC, Bittner D, Rome LH. Vaults. II. Ribonucleoprotein structures are highly conserved among higher and lower eukaryotes. Journal of Cell Biology 1990;110:895–901. doi:10.1083/jcb.110.4.895.