Development of vault-based nanovectors in *Pichia pastoris* and antibody-mediated targeting investigation on cancer cell lines.

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Abstract:

With a total molecular mass of 13 MDa, vault is the largest known ribonucleoprotein particle naturally occurring in higher eukaryotic cells[1]. 75% of the total protein mass is represented by 78 copies of the 97 kDa major vault protein (MVP) that assemble into a barrel-like “nanocapsule” enclosing poly(ADP-ribose) polymerase, telomerase-associated protein-1 and small untranslated RNAs. Vault complex is known to be involved in several cellular functions and many of its features make it a suitable nanovector for drug delivery.

Previously in our lab, recombinant vault has been produced by expression of the sole MVP sequence in insect cells, through the baculovirus-insect expression system. Due to low scalability and slow production rates of this system, the methylotrophic yeast *Pichia pastoris* (reported to enable vault expression at lower costs and in much higher yields[5]) has been chosen in this project to constitutively express human recombinant vaults. Vault has been purified by size exclusion chromatography following a protocol previously developed. Recombinant vaults from *Pichia* analyzed with transmission electron microscopy (TEM) show the same morphology and size of authentic vault.

Different cancer cell lines have the capability to endocyte the vault particle, at different extends[4]. In order to investigate vault antibody-mediated targeting to breast cancer cell lines, vault particles have been chemically conjugated with Cetuximab that binds the epidermal growth factor receptor (EGFR), overexpressed in these lines. Delivery and targeting have been monitored by confocal microscopy and flow cytometry analysis will follow in order to provide quantitative data.

Furthermore, this project aims to develop nanovectors based on Vault engineered variants. Recombinant vault containing a Cys-rich (CP) stabilizing domain has just been constructed. Further characterization by TEM, dynamic light scattering, and stability analysis of CP variant will be carried out.

[1] Kedersha, N. L., & Rome, L. H. (1986). Isolation and characterization of a novel ribonucleoprotein particle: large structures contain a single species of small RNA. *The Journal of cell biology*, *103*(3), 699–709.

[4] Galbiati, E., Avvakumova, S., La Rocca, A., Pozzi, M., Messali, S., Magnaghi, P., Colombo, M., Prosperi, D., & Tortora, P. (2018). A fast and straightforward procedure for vault nanoparticle purification and the characterization of its endocytic uptake. *Biochimica et biophysica acta. General subjects*, *1862*(10), 2254–2260.

[5] Wang, M., Kickhoefer, V. A., Rome, L. H., Foellmer, O. K., & Mahendra, S. (2018). Synthesis and assembly of human vault particles in yeast. *Biotechnology and bioengineering*, *115*(12), 2941–2950.