Development of a variant of vault nanoparticle optimized for antibody-mediated targeting

Pantaleoni C.[1], Cotugno D.[1], Tomaino G.[1,2], Valsecchi D.[1], Vanoni M.[1], Tortora P.[1], Frascotti G. [1]

E-mail: c.pantaleoni@campus.unimib.it

[1] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, 20126 Milano, Italy*

[2] *Université Paris Cité, CNRS, Institut Jacques Monod, F-75013 Paris, France*

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Abstract:

The vault protein is the largest known ribonucleoprotein particle, naturally occurring in higher eukaryotic cells. It is involved in several cellular functions. Many of its features, make it a suitable nano-vector of therapeutic molecules.

Here, the yeast *Komagataella phaffii* (ex *Pichia pastoris*) has been used to constitutively express human recombinant vaults, formed by 78 copies of the major vault protein (MVP) that assemble into a barrel-like “nanocapsule”. It has been subsequently purified by size exclusion chromatography. Purified recombinant vaults display the same morphology, size and biological properties than their natural counterpart, as shown by transmission electron microscopy, dynamic light scattering analysis and endocytic studies.

To achieve an antibody-mediated targeting to specific cell lines receptors, an engineered vault variant (vault-Z) was produced, by adding at the C-terminal of the MVP sequence a 33-amino acid peptide called Z peptide, derived from the bacterial protein A. This vault variant can specifically bind the constant region of human immunoglobulin G (hIgG), so it can be easily functionalized with monoclonal antibodies, which represent a general tool for selective targeting. The same technologies used to produce and characterize vault nanocapsule, were used for vault-Z; this particle retains the shape and dimensions of the authentic one. Fluorometric analyses demonstrated vault-Z particle’s binding to human labelled antibodies. Also, an in-depth characterization of the interaction mode between vault-Z and Trastuzumab, a human antibody that binds HER receptors on HER^+ breast cancer cell lines, is now in progress, alongside the assessment of the construct’s capability to target them. For this purpose, surface plasmon resonance, mass spectrometry and endocytosis analyses are being performed.