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PLANT HAIRY ROOTS AS BIOFACTORIES FOR THE PRODUCTION OF EXTRACELLULAR VESICLES WITH ANTITUMOR BIOACTIVITY

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Extracellular Vesicles (EVs) are nano-sized particles enclosed in a protein-lipid bilayer involved prevalently in cell communication. Plant EVs have been recently demonstrated to participate also in plant immunity and cell wall remodelling. Interestingly, plant EVs can be used as natural nanocarriers of bioactive molecules to human cells or as delivery tools for a next-generation drug delivery system. In addition, exploring the use of these EVs in the nutraceutical and pharmaceutical fields and their potential as drug delivery tools is an exciting perspective. However, one bottleneck for their use as therapeutics is the lack of standard protocols for plant EV production and the natural variability of their biomolecular cargo.

To overcome these issues, we explored the possibility to use plant hairy roots (HR) cultures as biofactories for the production of plant EVs. In particular, we have set up a reliable and reproducible procedure for purification and characterization of EVs released from HRs of medicinal plants, such as *Salvia dominica* and *Salvia sclarea*. We succeeded to isolate small EVs from the HR conditioned medium by differential ultracentrifugation. Particle size distribution and morphology of HR-

derived EVs were characterized by Dynamic Light Scattering (DLS), Nanoparticle Tracking Analysis (NTA) and Scanning Electron Microscope (SEM) showing that HR secrete round-shaped EVs ranging in size prevalently between 100 -200 nm. In addition, proteomic analysis of the HR-derived EVs revealed the presence of typical EV-associated proteins such as cytoskeletal components, chaperon proteins and integral membrane proteins, including the EV protein marker tetraspanin TET-7. Finally, we evaluated the impact of HR-derived EVs on the growth of HaCaT (human keratinocytes), MIA PaCa-2 (pancreatic carcinoma) and MCF7 (Breast cancer) cell lines. Our results showed that HR-derived EVs of S. dominica possess a strong and selective pro-apoptotic activity in cancer cells. Metabolomic analyses are in progress in order to identify bioactive molecules delivered by EVs and elucidate their selective mechanisms of action in cancer cells. Taken together, our results represents a significant step forward to the setup of a new plant biotechnology strategy for the purification of EV with therapeutic properties and a significant advance in EV research community