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SPATIAL PROTEOMICS RE-DEFINES PRECISE CARGOES OF EXTRA-CELLULAR VESICLES

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Virtually all cells secrete extra-cellular vesicles (EVs) containing a wide range of intracellular biomolecular material with key regulatory functions in cell-cell communication processes. The encapsulated content of EVs seems to reflect the cell of origin and, consequently, EVs have a huge potential as biomarkers for cancer and many other diseases. However, current approaches for purification of EVs possess inherent limitations and technical biases, resulting in heterogeneous preparations contaminated by other EVs subtypes and non-vesicular nanoparticles. This is especially problematic when specific regulatory functions are attributed to EVs subtypes.

Here, we report on the development of a spatial proteomic strategy enabling the deconvolution of the protein cargo of small EVs from non-vesicular particles. Crude EVs pellets (purified by differential ultra-centrifugation) are separated in a density gradient, analysed by LC-MS/MS and unambiguously assigned to different compartments by protein correlation profiling. Using this approach, we have systematically analyzed a panel of 14 cancer cell lines providing a highly curated catalogue of pan-human and ubiquitous proteins present in these vesicular entities. Our results have significant implications in the field of EVs, including the mechanisms of cargo selection. In addition, we demonstrate the applicability of our approach to analyze dynamic cellular responses and also to identify biomarkers in EVs purified from human biofluids.