Metabolically labeled exosomes for biogenesis and functional studies

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Exosomes are small Extracellular Vesicles (sEV) containing bioactive molecules, including proteins and nucleic acids, which make them a potent means of cellular communication. They are formed by an endosomal route by inward budding of the late endosome/multivesicular body (MVB) membrane. Despite in recent years much progress has been made to better define sEV composition and biogenesis pathways, their small size and heterogeneity pose challenges to find new reliable labelling strategies to identify specific exosome populations. We developed an innovative methodology to metabolically label fluorescent sEV through the use of a fluorescent lipid (Bodipy FL C16) that is readily internalized by cells and is transformed into phospholipids which will form part of the lipid bilayer of the secreted vesicles. Confocal microscopy showed colocalization with lipid transformation sites such as ER and mitochondria, and with specific markers of late endosomes or other organelles (tetraspanins, Golgi markers). Fluorescent sEV by melanoma cells were purified by differential ultracentrifugations, quantified by Flow Citometry (FC) and Nanoparticle Tracking Analysis (NTA) and sorted by Fluorescence Activated Cell Sorting (FACS). The secretion kinetic of Bodipy sEV showed an early release into the extracellular medium that reaches a plateau at about 6 hours. Bodipy sEV secreted in the conditioned media and purified by differential ultracentrifugation were separated by density gradient fractionation. Fractions analysed by FC displayed a single low-density peak that is detergent sensitive demonstrating that fluorescent particles are indeed lipid vesicles and contain tetraspanins (CD63, CD81 and CD9), syntenin and ESCRT components when analysed by Western Blot. Electron microscopy analysis of ultracentrifuged and sorted Bodipy sEV showed that Bodipy sEV have the typical shape and size (about 80 nm) of a subpopulation of sEV often referred to as small exosomes (Exo-S). Finally, colocalization studies of single Bodipy sEV with tetraspanins fluorescent antibodies showed colocalization of Bodipy sEV with CD63, CD81 and CD9. Taken together these results show an effective labelling of a discrete exosome population that can be further exploited for biogenesis and functional studies.