Effects of intracellular pathway inhibitors on the secretion, protein, and lipid composition of fluorescent Bodipy sEV

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Exosomes are small extracellular vesicles (sEV) formed within late endocytic compartments/multivesicular bodies (MVB). Various machineries have been described to regulate their biogenesis, including the ESCRT machinery, the syntenin–alix pathway, tetraspanins and lipids, but several aspects of these processes have not been fully elucidated. We developed a methodology to obtain fluorescent exosomes (Bodipy sEV) of endosomal origin by using Bodipy FL C16 (C16), a fluorescent palmitic acid that upon internalization by cells, is converted into phospholipids that are incorporated into the bilayer of secreted vesicles. These can be directly quantified by flow cytometry (FC). To gain insight into exosome biogenesis we combined this approach with the use of a panel of inhibitors of lipid metabolism and vesicular trafficking. Upon incubation of melanoma cells with C16 and selected inhibitors of cellular pathways, significant differences were observed in the secretion of Bodipy sEV as evaluated by FC and Nanoparticle Tracking Analysis, compared to the control cells. Interestingly, under all conditions, Bodipy sEV had the same relative distribution of tetraspanins (CD63, CD81, and CD9) as assessed by colocalization analysis. However, Western Blot analysis of sEV markers, highlighted significant differences. Additionally, phospholipid analysis revealed differences that could be attributed to the different metabolism of Bodipy lipids. In summary, our results indicate that the use of inhibitors of intracellular pathways not only affects the secretion of sEV, but also their protein and lipid composition. This suggests that this approach has the potential to provide further insights into the mechanisms underlying sEV production leading to a deeper understanding of their biological functions and future therapeutic applications for controlling EV release.