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Workflow to isolate and characterize extracellular vesicles derived from colorectal cancer cells

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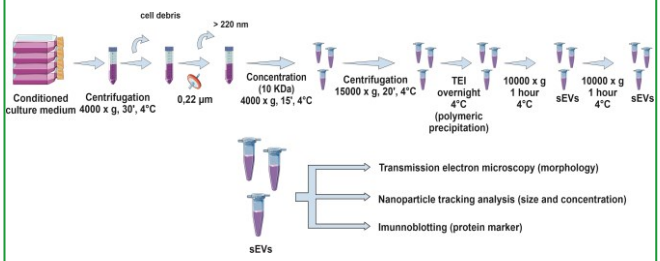
Introduction

Colorectal cancer (CRC) is in the top 10 cancers most prevalent worldwide, affecting equally men and women. More than 40 000 new cases of CRC will affect the Brazilian population in 2020/2022. Current research on tumor-derived extracellular vesicles (EVs) suggests that these small extracellular vesicles (sEVs) play an important role in mediating cell-to-cell communication and thus potentially modulating cancer progression via multiple pathways.

The main goal of this study was to elaborate a workflow to isolate and characterize EVs from the conditioned culture medium from two different colorectal cancer cells (HCT116 and HT29). The characterization of EVs follows the MISEV2018 guidelines.

Methodology

Conditioned medium from colorectal cancer cells were collected after 24 hours of incubation. EVs were isolated by ultrafiltration and polymeric precipitation. Morphology, size, concentration and protein markers were characterized in the EVs.



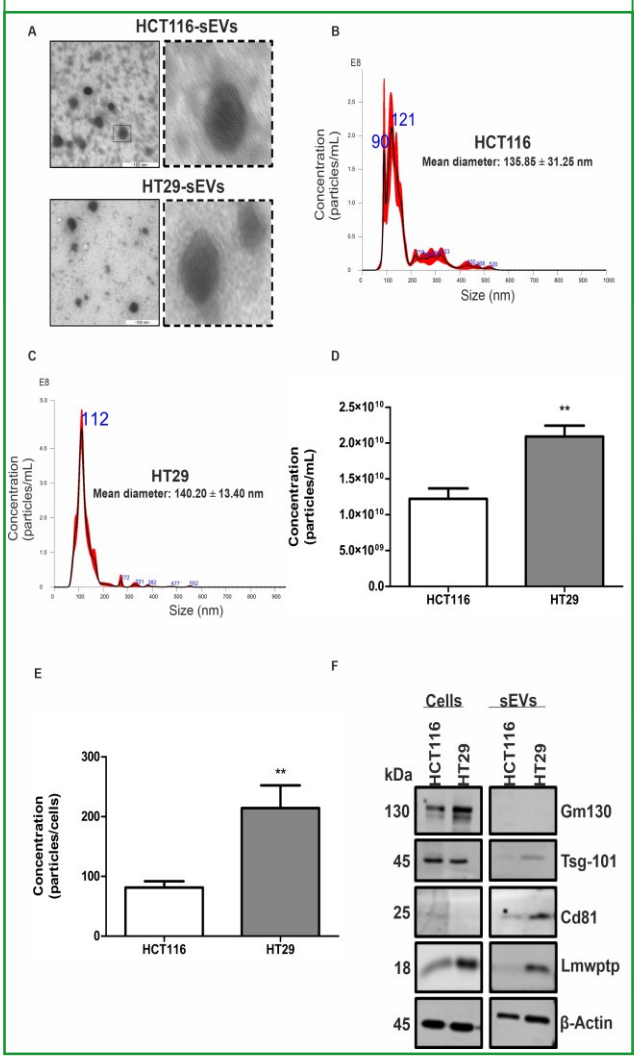
Results

We firstly focused on the characterization of EVs for size and purity (Figure 1), which was demonstrated by TEM that these vesicles displayed a compatible and expected morphology for sEVs (Figure 1A). NTA was used to characterize the size and estimated concentration of CRC cell-derived EVs.

As shown in Figures 1B, and C, the isolation protocol applied in this study, based on ultrafiltration and polymeric precipitation, purified a heterogeneous population of nanoparticles with mean diameters of 135.850 and 140.200 nm for HCT116 and HT29, respectively. According to the Minimal Information for Studies of Extracellular Vesicles 2018, the term small extracellular vesicles (sEVs) to describe vesicles smaller than 200 nm was used.

Results

A higher significantly concentration of sEVs was released by HT29 as compared with HCT116: $2.090x10^{10}$ vs. $1.223x10^{10}$ particles/ml ($p = 0.0021$; Figure 1D) even if we analyzed the ratio number of particles per cell: 214 vs. 81 ($p = 0.007$; Figure 1E). Our results showed that CRC cell-derived sEVs were positive for Cd81 and Tsg101 markers and negative for non-EV marker Gm130 (Golgi marker), confirming an enrichment in sEVs from the endocytic pathway in the samples (Figure 1F). We also observed that Lmwptp is a cargo of HT29-sEVs.



Conclusion

Our feasible experimental workflow effectively isolated and characterized small extracellular vesicles (sEVs) released from two distinct colorectal cancer cells. The combined methodology (ultrafiltration plus polymeric precipitation) reduced costs, time-consuming, allowing the isolation of significant yield of sEVs for further biological assays.

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