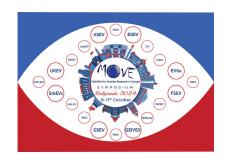


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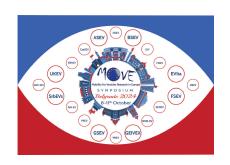








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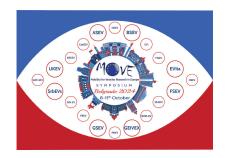


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Characterisation of thyroid-originating extracellular vesicles from thyroid cell culture medium and plasma of patients harboring thyroid tumors

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Introduction: Postoperative follow-up of differentiated thyroid carcinoma patients based on measuring thyroglobulin (Tg) levels is unreliable in patients with Tg autoantibodies (TgAt). Detecting thyroid markers on extracellular vesicles (EVs) might be a potent way to overcome these issues. We analysed the presence of CD63 (EVs marker) and thyroid-specific markers: thyroid-stimulating hormone receptor (TSHR) and Tg in EVs of thyroid cell lines to optimize detection and later in plasma EVs of thyroid nodule patients.

Methods: EVs were isolated by differential ultracentrifugation from thyroid normal (Nthy-ori-3-1) and cancer (TPC-1, OCUT2) cell lines and from patient's plasma (four benign, three malignant, and one recurrent). EVs number and size were measured with NTA. The presence of protein markers was determined using dot-blot.

Results: Nthy-ori-3-1 and OCUT2 cells were not viable after 6h of growth in serum-free medium, while TPC-1 cell line was viable until 24h, which influenced the number of isolated EVs. CD63 was present on EVs isolated from all cell lines. TSHR was confirmed in the cell lysate of all cell lines, while only EVs from TPC-1 harbored TSHR on their surface. Tg was present in the cell lysate and EVs originating from Nthy-ory-3-1 and TPC-1 cells, but not OCUT2. Marker analysis showed that plasma EVs from both malignant and benign groups were positive for CD63 and TSHR, and that they contain Tg, while levels of TSHR were higher in the malignant group. Plasma EVs from recurrent patient were positive for all three investigated markers.

Conclusions: TSHR found on the surface of thyroid-originating EVs from medium and plasma could allow immunoaffinity-based isolation of thyroid-specific EVs while the presence of Tg in these EVs holds a promise in detecting reliable Tg levels in TgAt recurrent patients. These promising findings need to be validated on a higher number of samples with analysis of Tg localisation.

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