A CONTINUOUS SPECTRUM OF VESICLE SIZES IS GENERATED THROUGHOUT THE COURSE OF APOPTOSIS

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Introduction: Although numerous aspects of apoptosis have been studied extensively, extracellular vesicles released during apoptosis, have received much less attention over the past decades.

Methods: In the present study we induced apoptosis of CCRF-CEM and U937 cell lines by staurosporine and etoposide, and focused on the released apoptotic vesicles. Apoptosis as well as primary and secondary necrosis were assessed by flow cytometry using Annexin V and propidium iodide staining at 0, 2, 4, 8, and 24 hours. Caspase activation was monitored by binding of fluorescent FAM-VAD-FMK to cells. Apoptotic cell derived vesicles were assessed by flow cytometry, transmission electron microscopy and tunable resistive pulse sensing techniques. Endoplasmic reticulum labeling was carried out by ER-Tracker staining.

Results: Both 2.5 μM of staurosporin and 50 μM of etoposide induced a simultaneous release of apoptotic microvesicles (<1 μm) and large apoptotic bodies (1 μm). In contrast to other extracellular vesicles and platelets (both being sensitive to detergent lysis), in the case of apoptotic vesicles Triton X-100 (≥ 0.02%) only abolished plasma membrane Annexin V binding, while forward and light scattering signals and propidium iodide staining were not abrogated. A higher proportion of large apoptotic bodies showed increased staining with propidium iodide and was found positive for ER-Tracker compared to apoptotic microvesicles.

Conclusion: The published literature distinguishes microvesicles from apoptotic bodies based on the arbitrary 1 μm size limit. Although vesicular structures below and above 1 μm were characterized by differential properties (including concentration, endoplasmic

reticulum content and propidium iodide staining), this work demonstrated a continuous spectrum of vesicle sizes released during apoptosis.

Bemutatás módja: **előadás**

Absztrakt témája: **elméleti**