

PHYSIOLOGICAL EFFECTS OF MELANOMA DERIVED EXTRACELLULAR VESICLES ON IMMUNE CELLS

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Keywords: extracellular vesicles, melanoma, cell migration

Almost all cells, including bacteria and eukaryotic cells are able to release extracellular vesicles (EVs). These small, membrane bound particles possess important role in intercellular communication. Among the EVs – based on their size, genesis and biological aspects – exosomes, microvesicles (MVs) and apoptotic bodies (ABs) are distinguished. Compelling evidence is gathered around the important roles of EVs in both physiological (e.g. immune response) and pathological processes (e.g. tumorigenesis), but these studies are mainly focused on tumour derived exosomes. However, information available on the effects of the other subtypes of EVs is limited.

Our aim was to compare the effect of primary (WM35) and metastatic (A2058) melanoma derived EVs on immune cells. We studied: (i) whether EVs are able to induce effector function (migration, phagocytosis) of the immune cells and (ii) molecular background of the cellular response detected.

As a model, A2058 and WM35 melanoma cell lines with different metastatic potential, Jurkat T-cells, THP1 and MonoMac6 monocytes were applied. The EVs were isolated from 24 hour supernatants using differential centrifugation and size exclusion chromatography (iZON qEV). For the characterization of EVs flow cytometry (BD FACSCalibur, Apogee Micro), dynamic light scattering (DLS) and mass spectrometry (MS) were used. The MS results were analysed in FunRich, with the help of UniProt, Panther and String databases. The EV-induced migration of the immune cells were evaluated in NeuroProbe chamber and HoloMonitor M4 microscope.

With flow cytometry and DLS we could characterise all the three subtypes of EVs produced by both primary and the metastatic melanoma cells. The melanoma derived MVs and ABs were proved to attach to the THP1 and Jurkat cells, but not to MonoMac6 cells. The A2058 derived MVs and ABs enhanced the migratory behaviour of Jurkat and THP1 cells. The A2058 derived MVs also induced morphological changes on the THP1 cells. However, the WM35 derived MVs induced migration on the THP1 cells, the ABs had no significant effect on cell motility.

In the MS analysis of the A2058 derived EVs 432 exosome-, 254 MV- and 294 AB-originated proteins were identified. Motility associated proteins identified were 131 in exosome-, 69 in MV- and 58 in AB-fraction. The cell adhesion molecules (e.g. ICAM1, ITGB3, ITGAV) were found: 37 in exosomes, 10 in MVs and 8 in ABs.

In conclusion, cell-specific activation, migration and morphological changes of immune cells were detected after melanoma derived EV-treatment. However, similar effects are induced by different EV subtypes and all contain cell-migration related proteins with different proportion, further analysis is required.