Melanoma-derived extracellular vesicles (EVs) as drivers of immunosuppression

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Background

- Evolution of metastatic melanoma from a primary tumor of the skin to widespread dissemination is crucially dependent on early regional lymph node metastases.
- Characterization of tumor-draining sentinel lymph nodes (SLNs) in patients reveals an immunosuppressed state amenable to tumor growth and progression prior to clinical evidence of nodal metastasis.
- The observation that regional immunosuppression is independent of nodal involvement suggests the lymphatic micro environment is altered prior to clinical evidence of metastasis and therefore an alternative mechanism independent of tumor cells is responsible for initiating this process.

Methods

- EV isolation and hypoxia: Melanoma (A375, C32TG, SK-MEL-28), Burkitt’s lymphoma (Daudi), and triple negative breast cancer (MDA-MB-231) cell lines were incubated for 96 hours with oxygen-exchanging culture flasks (normoxia) or low-oxygen transfer Petakali G3 flasks (Cartera) to induce hypoxia. The vesicles were purified from the supernatants using a water-excluding precipitation (Invitrogen, Life Tech) to enrich for membrane-bound EVs and size exclusion (Celartia) to induce hypoxia. The vesicles were purified from the supernatants using a water-excluding precipitation (Invitrogen, Life Tech) to enrich for membrane-bound EVs and size exclusion (Celartia) to induce hypoxia.
- EV proteomics: A total of 24 patient samples were analyzed for protein cargo.

Objectives

- In the current study, we evaluated melanoma-derived EVs for their potential to polarize immunity towards an immunosuppressive, tumor-promoting state.

Characterization of EVs

- EVs as Immune Modulators

EV Proteomics

- Lymphatic EVs in patients

Conclusion

- Under hypoxic conditions, increased EV production is observed in melanoma cell lines. EVs isolated from human plasma showed increased EV yield compared to cell line EVs irrespective of the presence of disease.
- Melanoma cell line EVs inhibit DC maturation and functional activation of T cells in a dose-dependent manner. Plasma-derived EVs are unable to inhibit DC maturation, suggesting these EVs are derived from a heterogeneous population of malignant and normal cells.
- Proteomic analysis of melanoma cell line EVs has identified candidate immunological and metastasis associated cargo. Culturing DCs with a subset of this cargo is insufficient for inhibiting DC maturation suggesting critical, synergetic interactions among the cargo proteins are required.
- EVs can be isolated from the lymph of affected lymphatic channels obtained from patients undergoing sentinel lymph node biopsy. We are currently exploring EVs as a viable communication mechanism between the primary tumor and sentinel lymph node.