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Introduction: Various mammalian cells, including cancer cells, shed extracellular vesicles (EVs) into the surrounding tissues. Although mammalian EVs play multiple roles in the tumour growth and metastasis by promoting angiogenesis, the biological functions of tumour tissue-derived EVs *in vivo* have never been investigated. Here, we isolated and characterised the mouse melanoma tissue-derived EVs, and investigated their roles in neovascularisation. *Materials and methods*: EVs were isolated from mouse melanoma tissues by serial centrifugation and density gradient ultracentrifugation. These purified EVs were analysed by electron microscopy, dynamic light scattering and Western blotting analyses. Mouse Matrigel plug assay was carried out to examine the pro-angiogenic activity of the EVs. *Results*: Isolated EVs from mouse melanoma tissues were closed lipid-bilayered vesicular forms with ~150 nm size in diameter. These EVs were enriched with vesicular marker proteins (CD9, CD81 and Tsg101), tyrosinase, F4/80, alpha-smooth muscle actin and E-selectin, suggesting that melanoma cell-, macrophage-, fibroblast- and endothelial cell-derived EVs are present in the melanoma microenvironment. When tumour-derived EVs within Matrigel were injected subcutaneously into mice, a massive formation of CD31-positive vessel-like structures and infiltration of F4/80-positive macrophages were observed. We observed that EVs harboured VEGF and significant amount of VEGF was detected in EV-treated Matrigel. Moreover, SU5416 (a VEGF inhibitor) almost completely blocked EV-induced macrophage infiltration and angiogenesis. *Conclusions*: This study showed that both cancer cell- and stromal cell-derived EVs were present in the tumour microenvironment. These tumour tissue-derived EVs have VEGF-dependent angiogenic activities. Further studies should be warranted to decipher the molecular mechanisms involved in the role(s) of EVs in tumour growth, angiogenesis and metastasis.

Ovarian cancer cell-secreted exosomes induce molecular and phenotypic changes in cells

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Ovarian cancer is the fifth most deadliest cancer amongst women in the United States, and the most lethal gynaecological malignancy in the world. Recent studies reveal that human tumour cells release cell-secreted vesicles called exosomes. Exosomes are endosome-derived vesicles containing bioactive materials, including miRNAs that can be detected in the bloodstream and urine. Importantly, stem cell factor LIN28, a regulator of let-7 miRNAs, is present in ovarian cancer cells. Our preliminary data revealed a potential regulatory role of LIN28-let-7 miRNA in ovarian cancer cells, that may play a role in cancer metastasis via their secreted exosomes. We hypothesised that ovarian cancer cell-secreted exosomes are taken up by target cells and induce changes in gene expression and cell behavior. Our objectives were to: (a) determine the effects of IGROV1 cell-secreted exosomes on HEK293 cells, (b) identify genes related to the epithelial to mesenchymal transition (EMT) pathway, that are modulated in HEK293 cells following exposure to IGROV1-secreted exosomes and (c) identify miRNAs present in IGROV1-secreted exosomes, that are predicted to target genes involved in EMT. Our data revealed that IGROV1-secreted exosomes are taken up by HEK293 cells. In addition, HEK293 cells treated with IGROV1-secreted exosomes had increased levels of LIN28, and demonstrated increased invasion and migration ($p < 0.04$). Finally, various genes involved in EMT, including TIMP1 (25-fold higher), FOXC and NOTCH1 (11-foldhigher), CDH1 (6-fold higher), MMP2 (5-fold higher), MMP9 (4-fold higher) and ZEB1 (3-fold higher) were up-regulated in HEK293 cells, that have taken up IGROV1-secreted exosomes. Elucidating the molecular and phenotypic effects that ovarian cancer cell-secreted exosomes have on non-cancerous cells, will lead to greater understanding and insight into cancer metastasis and tumour development.

Prostate cancer derived exosomes could promote prostate cancer progression via activation of the ERK cell signalling pathway

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Introduction: Prostate cancer (PCa) is the leading diagnosed and third most lethal malignancy in men. During early stages of the disease, when confined to the prostate, it is androgen-dependent, and yet curable by surgical intervention or radiation treatment. Cancer which has metastasised may be treated using hormone withdrawal therapy; however, over time many PCas invariably became resistant to treatment and progresed to Castration Resistant Prostate Cancer (CRPC), which ultimately results in death. While numerous treatment resistance mechanisms have already been identified, understanding factors contributing to CRPC continues to be an important research focus. Exosomes are nanometer sized cup-shaped membrane vesicles which are secreted from normal and cancerous cells. Exosomes could play a significant role in paracrine signalling pathways, thus potentially influencing cancer progression. We explored the ERK pathway in exosomes since it has the potential to influence a wide variety of cell functions, as diverse as cell proliferation to apoptosis. *Methods*: Exosomes were purified from the conditioned media of different prostate cell lines using differential centrifugation, followed by an ultracentrifugation step in 30% sucrose. Further analysis using Western blot (WB) and transmission electron microscopy validated the purity and integrity of isolated exosomes. Alterations in the ERK cell signalling pathway in recipient cells were then studied, upon exposure to exosomes derived from a panel of prostate cell lines. *Results*: Our WB data confirmed a pure enrichment of exosome markers in the exosome isolate. WB data also indicates that exosomes derived from PCa cell lines will activate ERK phosphorylation and subsequent signalling via Ras/Raf/MEK pathway. *Conclusion*: The Ras/Raf/MEK/ERK signalling pathway may be activated in a panel of prostate cells by exosomes derived from PCa cell lines. Exosomes could, therefore, influence signalling pathways involved in PCa progression.

Role of interleukin 8 in exosome-mediated crosstalk between chronic myelogenous leukaemia cells and bone marrow stromal cells

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Introduction: Chronic myelogenous leukaemia (CML) is a myeloproliferative disorder characterised by t(9:22) (q34:q11) reciprocal translocation, resulting in the expression of chimeric Bcr–Abl oncoprotein with constitutive tyrosine kinase activity. Exosomes (Exo) are small vesicles of 40–100 nm in diameter released by many cell types including cancer cells that have a role in cell-to-cell communication and tumour–stroma interaction, thus potentially affecting cancer progression. It is well known that stromal microenvironment contributes to disease progression through the establishment of a bi-directional crosstalk with cancer cells. In bone marrow, stromal cells are able to sustain the growth and survival of leukemic cells by protecting malignant cells from chemotherapy-induced death; contrarily, leukaemia cells induce changes in the bone marrow architecture. Our hypothesis is that exosomes could have a functional role in this crosstalk. *Materials and methods*: Cell lines used in experiments are LAMA84, a human CML cell line, and HS5, a bone marrow derived human stromal cell line. Quantitative gene expression analysis for IL8, IL6 and VEGF was performed by TaqMan RT-PCR; colony formation assay was performed with methylcellulose; adhesion assay was performed with LAMA84 and HS5 cell lines; Western blot analysis was performed with antibodies anti-Akt, anti-pAkt, anti-Erk 1/2 and anti-p-Erk 1/2. *Results*: Treatment of HS5 cells with LAMA84-derived Exo induced a significant increase of IL8 as well as LAMA84 cell adhesion to stromal monolayer.

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