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Abstracts

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selectively released in exosomes and 14 were selectively retained in cells. Comparing across all cell lines, we noted that miRNAs 223-3p, 142-3p, 451a, 144-3p and 150 were up-regulated in 5/5 exosome samples tested, and 145-5p and 605-5p were found to be up-regulated in 4/5 exosome samples relative to the cells they were derived from. The miR-502-5p was the only miRNA found to be selectively retained in 5/5 cell lines relative to the exosomes. The miRNAs up-regulated in exosomes regulate key oncogenes, including EGFR and c-Myc. Exosomal miRNAs were enriched for a known RNA binding motif UGUA. *Summary/conclusion*: We have identified a set of miRNAs that are commonly enriched in lung adenocarcinoma cell line exosomes. These miRNAs appear to regulate key oncogenes in lung adenocarcinoma. The discovery of novel exosomal miRNA function and mechanisms could directly impact patient care through the development of novel therapeutics.

P-XII-4

Extracellular vesicles as a potential mediator of microRNA-linked ovarian cancer drug resistance

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Introduction: Ovarian cancer is the deadliest gynaecological cancer. A major contributor to the poor survival rate is the development of chemoresistance to platinum-based therapies such as cisplatin and carboplatin. We aimed to test the role of miRNAs in the acquisition of drug resistance in ovarian cancer and whether vesicular transfer between cells could be a route by which resistance can develop. *Methods*: We used microarrays to measure miRNA levels in the ovarian cancer cell line A2780 and its cisplatin-resistant derivative CP70. The role of miRNAs and mRNA targets were tested using transfected miRNA mimics and siRNAs, respectively. CP70-derived extracellular vesicles were added to cisplatin sensitive A2780 cells, and the effect on drug resistance was measured. Delivery of miRNAs was assessed by qRT-PCR. *Results*: We identified several miRNAs that are increased in cisplatin-resistant cells. We show that most of these do not directly contribute to cisplatin resistance. Interestingly, miR-21-3p, the passenger strand of the known oncomiR, directed increased resistance to cisplatin in a range of ovarian cell lines. This effect was specific to the star strand, as miR-21-5p had the opposite effect and actually increased sensitivity of A2780 cells to cisplatin. We identify NAV3 as a potential target of miR-21-3p and show that knockdown of NAV3 increases resistance. Extracellular vesicles released by CP70 cells were also capable of increasing resistance in A2780 cells, which may be contributed by the delivery and increase in miR-21-3p. Finally, we use publically available transcriptomic data to demonstrate that miR-21-3p is raised, whilst NAV3 is reduced, in ovarian tumours that are resistant to platinum treatment. *Summary/conclusion*: Our data suggest that miR-21-3p can induce cisplatin resistance in ovarian tumours, potentially by targeting the NAV3 gene, which could be promoted by the localized release of drug-resistant cell-derived extracellular vesicles.

P-XII-5

Curcumin induces selective packaging of miR-21 in exosomes released by chronic myelogenous leukaemia cells

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Introduction: Chronic myeloid leukaemia (CML) is characterized by the clonal expansion of myeloid precursors. Exosomes are nanovesicles able to modulate intercellular communication and tumour microenvironment. Exosomes contain miRNAs that can influence gene expression in target cells. The miRNAs, such as miR-21, with tumour-suppressor functions are often lost in cancer. Some observation indicate a possible cellular disposal of miRNAs by exosomes. Curcumin affects the expression of microRNAs in CML cells and according to our data may play a role in this process. *Methods*: Exosomes were collected by K562 and LAMA84 conditioned medium by ultracentrifugation. CML cells were treated with Curcumin. The miR-21 expression, PTEN and VEGF mRNA were assessed by real time PCR. VEGF secretion and pAKT were evaluated by ELISA. The anti-cancer effects of Curcumin, in vivo, were evaluated with a xenograft CML tumour model. *Results*: Nanovesicles of CML cells treated with curcumin were characterized by physical and biochemical methods. DLS analyses indicated that isolated exosomes had an average size of 80 nm and contained Alix and TSG 101. Curcumin treatment caused miR-21 decrease in CML cells, but a greater amount was observed in exosomes. In order to support our hypothesis that decrease of miR-21 was determined by a selective enrichment of this miRNA in CML exosomes, we treated CML cells with GW4869, an inhibitor of exosome release. GW4869 treatment induced an increase of miR-21 in CML cells compared with curcumin-treated cells. The addition of curcumin, to CML cells, caused a dose-dependent increase in PTEN, well-known target of miR-21, at mRNA and protein level. Curcumin treatment decreased AKT phosphorylation and VEGF expression. The effects of curcumin on a xenograft CML tumour model, confirmed the in vitro results and the anticancer effects of curcumin. *Summary/conclusion*: Our data suggest that curcumin caused a decrease of miR-21 in CML cells through a selective packaging of miR-21 in exosomes.

P-XII-6

Identification of specific miRNA expression pattern in exosomes of invasive urinary bladder cancer cell lines

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Introduction: Interaction of tumour cells and tumour microenvironment (TME) plays an important role in tumourigenesis and progression. Thereby, exosomal microRNAs (miRNAs) can affect cell–cell communication at the site of origin as well as the TME. The aim of the project is the identification of a specific miRNA expression pattern from in-vitro obtained tumour-derived exosomes of urinary bladder cancer (UBC) cell lines in correlation to their malignant potential. Furthermore, we want to analyze the effect of these exosomal miRNAs on tumour-associated fibroblasts (TAFs). *Methods*: Exosomes were isolated from invasive (T-24, 253J-BV and J82) and non-invasive (RT-112, 5637) UBC cell lines. The number and size of vesicles were measured by NTA. Exosomal and contamination markers were analyzed by Western blotting. Total RNA was isolated from cells and their exosomes (upon treatment with RNase). MiRNA expression pattern of UBC cells and exosomes was analyzed using miRNA microarray and qPCR. Exosome-mediated miRNA transfer between cancer cells and TAFs was verified by 1) transfection of donor UBC cells with *non-human* miRNA, cel-miR-39, 2) Exosome isolation and RNAse treatment, 3) Transfer to recipient TAFs and 4) qPCR analysis using total RNA from the recipient TAFs. *Results*: The isolated exosomes exhibited a high amount of exosomal markers (CD63, CD81, syntenin). Sixteen miRNAs were identified, which distinguish invasive UBC cells from non-invasive cells. Exosomes secreted by invasive UBC cells are characterized by a specific miRNA signature of 25 miRNAs. Six differently expressed miRNAs were validated by qPCR. After successful transfection of RT-112 and T-24 with cel-miR-39, cel-miR-39 was detected in RT-112 and T-24 exosomes as well as in recipient TAFs cultivated in the presence of these exosomes. *Summary/conclusion*: Exosomes secreted by UBC cells exhibit a specific miRNA signature depending on the invasive