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Abstracts

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Poster Presentations

Arcadis Room
Poster Session 1A - EV in the tumor micro-environment
Chair: *Clark C. Chen and Janusz Rak* 13:00-14:00

P1A-018

Role of tumour-derived exosomes in growth and metastasis of cancers

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Introduction: Cancer burdens and death are largely due to metastasis in several cancer types, including lung and breast. The pervasive mortality from metastasis highlights the shortcomings of traditionally accepted hypotheses on the metastatic process. Exosomes are biological nanovesicles (30–100 nm) of endocytic origin that mediate local and horizontal transfer of information. Studies indicate tumour-derived exosomes (TDEs) as critical mediators of tumourigenesis and metastasis. Here, we examine the role of TDEs in lung and breast cancer metastasis. *Methods*: Non-metastatic lung (H522) and breast cancer (T47D) cells were treated with fluorescent dye (PKH67) labelled exosomes from metastatic lung (H1299 and A549) and breast (MDA-MB-231) cancer cells, respectively. Changes in growth rate, migratory and invasive behaviour were assessed. EMT-associated proteins were analysed by western blot. Metastasis-associated miRNAs were analysed by RT-PCR. *Results*: Our findings suggested: (a) TDEs had a mean size of 92 nm and carried exosomal marker proteins CD63 and CD81, (b) uptake of exosomes from highly metastatic cancer cells by non-metastatic recipient cells, (c) faster wound healing rate, increased migratory behaviour, and invasiveness in recipient cells, (d) increased endothelial cell tube formation, reflective of angiogenesis ability of TDEs, (e) modulation in expression levels of key EMT-associated in non-metastatic cells treated with TDEs favouring EMT shift, (f) difference in metastasis-associated miRNAs levels in exosomes from invasive and non-invasive cancer cells. *Summary/conclusion*: Exosomes from metastatic cancer cells carry the cargo essential for EMT and have the ability to change phenotypic traits of the recipient cells. Understanding the role of TDEs in cellular process would enable better management of cancer metastasis and will have great potential to uncover new targets and lead to new therapies for metastatic disease.

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P1A-019

Role of miR-126 shuttled by exosomes in the crosstalk between chronic myelogenus leukaemia and endothelial cells

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Introduction: Exosomes are nanovesicles that mediate intercellular communications and modulate tumour microenvironment. Our previous work showed that exosomes released by myeloid chronic leukaemia cells, LAMA84, stimulate angiogenesis, in vitro and in vivo. Because exosomes are involved in horizontal transfer of information through the export of miRNAs, we focused on the role of miRNAs on crosstalk between CML cells and endothelium. *Methods*: Exosomes were collected by LAMA84-conditioned medium by ultracentrifugation. miRNAs profiling was performed by miRNA-array and RT-PCR. HUVECs, transfected or not with miR-126 mimic or inhibitor, were treated with exosomes. CXCL12 and VCAM1 mRNA were assessed by RT-PCR. CXCL12 secretion was evaluated by ELISA. Biological effects of miR-126 shuttled by exosomes in HUVECs were evaluated by motility and adhesion assays. *Results*: Exosomes released by LAMA84 transport microRNAs. Among of 124 miRNAs identified in LAMA84-Exo, we focused our attention on miR-126. This miRNA was upregulated in exosomes respect to cells and targets CXCL12 and VCAM1. We transfected LAMA84 with labelled miR-126-Cy3 and leukaemia cells were co-cultured with HUVECs. miR-126-Cy3 was shuttled to endothelial cells. The treatment of LAMA84 cells with GW4869 blocked this transport. As the treatment of HUVEC with LAMA84-exo for 24 h reduced CXCL-12 and VCAM-1 expression, we ascribed these effects to exosomal miR-126 internalized by endothelial cells. By luciferase activity assay, we confirmed that exosomal miR-126 targets CXCL-12 and VCAM-1 3'UTR mRNA in HUVECs. Moreover, we observed that reduced levels of CXCL-12 and VCAM1 affect negatively LAMA84 motility and cells adhesion. MiR-126 inhibitor reverted the decrease of CXCL12 and restored the motility and adhesion of LAMA84. Over-expression of miR-126, with miR-126 mimic, showed opposite effects. *Summary/conclusion*: Our data suggest that CML-exosomes facilitate mobilization of leukemic blast from bone marrow and their diffusion in the bloodstream.

P1A-020

Lung cancer-derived extracellular vesicles promote cancer progression by triggering oncogenic signals and increasing vascular permeability in an autocrine/paracrine fashion

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Introduction: Extracellular vesicles (EVs) have been shown to play important roles in many diseases including tumour. However, the role of EVs in lung cancer is still largely unknown. In this study, we tried to find out the biological functions of EVs in lung cancer and evaluate the clinical applications. *Methods*: EVs were isolated from culture supernatants, serum, and malignant pleural effusion (MPE) using ultra-centrifugation and ultra-filtration and then evaluated by TEM, cryo-EM, Nanosight, and western blotting. The biological functions of EVs were analysed in both in vitro cell line model and in vivo animal model. *Results*: EVs could be isolated from culture supernatants, serum, and MPE samples using both these two methods with different capacity revealed by EM and Nanosight. Specific EV markers including Alix, CD63 and, Tsg101 were detected

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in the isolated EVs. The EVs carried various RNA species that small RNAs seemed to be enriched. Furthermore, the EVs could be uptaken by lung cancer cells and trigger oncogenic signals such as Stat3 and Akt. Previously, we have shown that IL-6/Stat3/VEGF pathway plays an important role in lung cancer angiogenesis and metastasis. Here, we showed that EVs from lung cancer samples carried high level of VEGF and triggered vascular permeability changes in mice. *Summary/conclusion*: Using these methods, we isolated EVs not only from culture supernatants but also various lung cancer-associated clinical samples. Furthermore, the EVs could promote cancer progression by triggering oncogenic signals and increasing vascular permeability in an autocrine/paracrine fashion. These results may help the understanding of the biological functions of EVs in lung cancer and also the discovery of novel biomarkers and potential drug targets.

P1A-021

Crosstalk between chronic myelogenous leukaemia and bone marrow stromal cells: role of exosomes in the il8-dependent signalling mediated by egfr activation

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Introduction: Chronic myelogenous leukaemia (CML) is a myeloproliferative disorder characterized by Bcr-Abl oncoprotein with a constitutive tyr-kinase activity. Exosomes (exo) shed by cancer cells potentially affect tumour–stroma interaction through the establishment of a bi-directional crosstalk. Interleukin-8 (IL8) is a pro-inflammatory chemokine that regulate proliferation and survival of cancer cells. We previously demonstrated that CML-derived exo modulate bone marrow microenvironment through the IL8 secretion from stromal cells. EGFR, as well as IL8, regulate cell proliferation and survival; it has been recently demonstrated that EGFR ligands can signal via exosomes shed by cancer cells. We hypothesized that the effects induced by IL8 are EGFR mediated and exosomes are involved in this pathway. *Methods*: Human cell lines used are LAMA84 (CML cells) and HS5 (stromal cells); gene expression analysis was performed by RT-PCR and western blot with antibodies for EGFR, pEGFR and AREG. For in vivo experiments, LAMA84 cells were inoculated in NOD/SCID mice and treated with IL8, vehicle (PBS), SB (IL8 receptors inhibitor) or IL8 + SB. After 50 days, tumours were removed to calculate their weights and RNA was extracted from biopsies. *Results*: On the basis of the in vitro effects of IL8 on LAMA84 cells, we studied the in vivo role of this cytokine. Mice treated with IL8 develop larger tumours than control groups; co-treatment with SB resulted in a slower tumour growth compared with mice treated with IL8 alone. The expression of pro- and anti-apoptotic genes confirmed a pro-survival role of IL8 in vivo. LAMA84 cells and their exo showed different EGFR ligands. Exo treatment of stromal cells increases EGFR expression possibly inducing activation of survival pathways mediated by IL8. *Summary/conclusion*: Our data show that IL8 promotes tumour growth and survival in vivo. Exo, carrying EGFR ligands, modulates bone marrow microenvironment through activation of EGFR signalling on stromal cells, demonstrating a new extracrine signalling mediated by EGFR ligands.

P1A-022

Role of exosomes released by colon cancer stem cells in the modulation of tumour microenvironment

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Introduction: Colon carcinoma is characterized by a heterogenic pool of cells with distinct differentiation patterns. It was reported that a population of undifferentiated cells from the primary tumour, called cancer stem cells, can reconstitute the original tumour on xenotransplantation. Colon cancer stem cells (CCSCs) are able to initiate and sustain tumour growth. Recently it was reported that renal cancer stem cells released exosomes that stimulate angiogenesis and pre-metastatic niche formation. Exosomes are nanosize vesicles derived from endosomal compartment released in extracellular space. Exosomes contain proteins, mRNA, and microRNAs and function as mediators in cell-to-cell communication. We characterized vesicles released by CCSCs and investigated on their involvement in angiogenesis and in pre-metastatic niche formation. *Methods*: Exosomes were collected from condition medium of CCSCs grown as sphere (Sphere) and SDAC (sphere-derived adherent cells). Vesicles were characterized by nanoparticles tracking analyses (NTA), western blotting and enzymatic assay. HUVECs were treated for 6 h with exosomes, gene expression was analysed with RT-PCR and protein secretion was evaluate by ELISA. *Results*: CCSC released vesicles that enriched in Alix and acetylcholinesterase. NTA showed that the peaks of particle size were approximately 100 nm for Sphere and 60 nm for SDAC, according with the expected size of exosomes. CCSCs–exosomes were internalized by endothelial cells, and they modulated IL8, IL6, VCAM1 and CXCL12 mRNA expression. Exosomes induced CCSC adhesion to endothelial cells pre-treated with CCSCs–Exo. In vitro and in vivo angiogenesis assay showed that exosomes induced neovascularization. *Summary/conclusion*: CCSCs–Exo are involved in modulation of tumour microenvironment, in particular they induced an angiogenic phenotype in endothelial cells. Work is in progress to investigate if CCSCs–Exo are able to modulate the pre-metastatic niche formation.

P1A-023

Modification of protein and microRNA profile in extracellular microvesicles by UPR in CML cells

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Introduction: There is a growing need to determine the role of the microenvironment in regulation of growth, self-renewal and drug resistance of leukaemic cells in the bone marrow, as it seems that the communication of leukaemic progenitor and stroma cells might play an important role in the cancer progression. Secretome, which bypass the information and regulatory signals by the usage of proteins and microRNA, plays a crucial role in this crosstalk. Oncogene activity leads to deregulation of various signalling pathways. Our recent data showed that BCR-ABL1 activity in CML cells leads to activation of the unfolded protein response (UPR) (Kusio-Kobialka *Cell Cycle* 2012). This results in rearrangement of mRNA translation, thus allowing for adjustment of cellular proteome and adaptation of cancer cells to stress conditions. The aim of this study was to verify if composition of the microvesicles secreted by CML cells might be modified respectively to the activation of UPR and how it influences MVs properties. *Methods*: Using mass spectrometry analysis, we compared which proteins are present in mouse 32D cells-parental and expressing BCR-ABL1 as well as in human K562 CML cell line. Profile of microRNAs secreted in microvesicles by K562 cells was determined using microarrays and confirmed by real-time PCR analysis. The influence of isolated microvesicles either from bone marrow stroma fibroblasts or CML cells conditioned medium on proliferation and apoptosis of targeted cells was assayed using multiparameter flow cytometry. *Results*: Bioinformatic analysis indicated that most of the proteins identified in the CML microvesicles are known to play role in the cell movement, modification of extracellular matrix and intercellular signal transduction. We experimentally confirmed that presence of CML microvesicles facilitates cells motility and invasiveness in matrigel invasion assay. Furthermore,