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Abstracts

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Background. It has recently been shown that microvesicles derived from activated T cells can stimulate human mast cells. This pattern of activation involved the MAPK system and resulted in degranulation and the release of several cytokines such as IL-8 and oncostatin M (J Immunol 2010;185:4206). To characterise this novel pathway of mast cell activation, we analysed the specific gene expression profiling by microarray analysis and identified that this stimulation leads to the production of several cytokines and chemokines, here-to-fore unknown in mast cells, such as IL-24. *Methods*: T cell-derived microvesicles were labelled with PKH67 to allow visualisation of their interaction with human mast cells. Consequent gene expression profiling was studied by whole genome microarray and analysed for the identification of cellular pathway clusters. Expression of three selected genes, CCL3, CCL7 and IL-24, was validated by qRT-PCR and specific ELISA. IL-24, that has not been here-to-fore recognised in mast cells, was also tested for its effect on keratinocyte STAT3 phosphorylation and for its presence in mast cells in psoriatic skin lesions. *Results*: The uptake and internalisation of T cell-derived microvesicles into human mast cells occurred within 24 hrs. This led to the robust upregulation of several clusters of genes, notably those that are cytokine-related. Amongst these, IL-24 appeared to be a hallmark of microvesicle-induced activation. Mast cell-derived IL-24, in turn, activates keratinocytes *in vitro* as manifested by STAT3 phosphorylation and is produced in mast cells within psoriatic lesions. *Conclusion*: Production of IL-24 is a unique feature of microvesicle-induced mast cell activation, as its production by these cells has not been recognised so far. We propose that this mast cell-derived cytokine may contribute to the pathological findings in T cell-mediated skin inflammation.

Phenotype and function of γδT cell exosome-like vesicles: potential for therapeutics?

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Introduction: A small subset of unconventional human T cells, γδT cells, play complex roles in host immunity against pathogens and cancers. A key function is antigen presentation, comparable to that of dendritic cells. These cells are easily expandable and potentially useful therapeutically. Here, we characterise the phenotype and function of activating chemokine secretion by antigen-specific αβCD8+ T cells. Future work will evaluate and compare the potency of these exosome-like vesicles with other sources of T cell activation, as well as their potential to be therapeutically valuable.

Exosomes released by chronic myelogenous leukemia cells modulate γδT cell activities

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Introduction: Exosomes are small vesicles of 40–100 nm diameter of endosomal origin which are secreted from different cell types, including cancer cells. Several data from the previous years have showed that exosomes are messengers in intercellular communication and that tumour cells can use these vesicles to affect immune function. Some reports detail lymphocyte activation following tumour–exosome interactions while others describe immune-suppressive effects. The ability of tumour cells to evade or suppress an active immune response is considered to be a significant factor in the development and progression of tumours. We studied the effects of exosomes released by Chronic Myelogenous Leukemia (CML) cell line, K562, on γδT cell activities, to better understand the interaction between cancer-exosomes and the immune system. *Materials and methods*: Exosomes were isolated and purified from K562 cells by ultracentrifugation of conditioned culture medium. Exosomes were added to γδT cells at different doses and analysed for NKG2D, CD69, CD25, IFNγ and TNFα. To determine proliferation and apoptosis, γδT cells were stained for CFSE and Annexin V. *Results*: We demonstrate that CML-derived exosomes are able to downregulate NKG2D receptor, CD69/CD25 activation marker and impair the ability of γδT cells to produce IFN γ and TNFα, suggesting their inhibitory effect on lymphocytes function. Finally, we show that addition of exosomes to γδT cells impair their proliferation and induce apoptosis. The suppressive activity of K562 exosomes was susceptible to inhibition with SB-431542 and involved a crucial role of the TGFβ, suggesting that TGFβ probably might be the main immunosuppressive cytokine present in our exosome preparation responsible for the observed antiproliferative and inhibition effect. *Conclusions*: These findings suggest a role of CML exosomes in the modulation of γδT cells function and increase our knowledge on exosomes-mediated immune-escape mechanism.

Effect of human melanoma exosomes on the function of antigen-specific CD8+ T cells

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increases the adhesion of leukemic cells to stroma. The inhibition of IL8 receptors, CXCR1 and CXCR2, on LAMA84 cells revert the effects described previously. *Conclusions*: Our data show that LAMA84-derived Exo modulate bone marrow microenvironment, increasing the production of the IL8 on stromal cells; moreover IL8 is able to affect leukaemia cell proliferation and survival in a paracrine fashion.

Characterisation of extracellular vesicles in chronic lymphocytic leukaemia

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Introduction: The pathogenesis of chronic lymphocytic leukaemia (CLL) is stringently associated with a tumour-supportive microenvironment. Of note, CLL cells themselves initiate promotive responses in surrounding cells, and Extracellular Vesicles (EVs) released by CLL cells represent a newly discovered mechanism of cell communication. Thus, we aim to characterise EVs present in blood plasma of CLL patients and released by primary CLL cells in culture in order to understand their role within microenvironment. *Material and methods*: EVs were isolated from blood plasma of CLL patients and healthy donors and also from supernatant of primary CLL cells in culture. Purification of EVs was performed by serial centrifugation and density-based separation. RNA and protein lysates of EVs and respective cells of origin were analysed via Bioanalyser profiling and quantitative RT-PCR or via Coomassie-staining of SDS-PAGE gels and mass spectrometry, respectively. *Results*: We were able to isolate EVs from blood plasma of CLL patients and healthy controls. Protein profiling comparing lysates of EV and peripheral blood mononuclear cells from same donor revealed an EV-specific protein profile. Proteins present in EVs include fibronectin precursor, CD36, MHC class I proteins and heat shock proteins. In order to characterise CLL-cell derived EVs, we established different culture systems of CLL cells, which mimic microenvironmental niches in CLL. Analyses regarding the amount of EVs produced as well as the RNA and protein composition are currently conducted. Of note, first results in Mec1 cells, a CLL cell line, revealed an enrichment of small RNAs and shuttling of microRNA-155 in EVs. *Conclusion*: CLL cell-derived EVs might be involved in the establishment of a promotive microenvironment. Thus, their characterisation will help to identify EV-associated molecules involved in CLL pathogenesis, with the final goal to develop new therapeutic options for CLL.

Microvesicles and epithelial mesenchymal transition in the development of cancer

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from PNT2 control cells and MV-treated cells were profiled by SDS-PAGE for identification by mass spectrometry. *Conclusions*: The correlation between EMT and malignancy has been well documented in almost all carcinomas of epithelial origin. EMT basically explains how epithelial tumour cells can escape from primary residence, travel to distant sites and establish secondary tumours? Our work is the first report of EMT on normal prostate cell lines induced by microvesicles.

Role of microRNAs shuttled by exosomes in the crosstalk between chronic myelogenous leukaemia and endothelial cells

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Introduction: Exosomes are membranous nanovesicles derived from endosomal membrane compartments that are released from cell surface following activation or stimuli. Exosomes contain proteins, mRNA and microRNAs (miRNAs) and function as mediators in cell-to-cell communication. Exosomes can be transported within different cells thus affecting phenotypes of the recipient cells. A number of studies have described exosomes as new players in modulating tumour microenvironment, promoting angiogenesis and tumour development. Neovascularisation is known to exert an important role in the progression of chronic myelogenous leukaemia (CML). CML is a myeloprolifetive disorder characterised by the presence of Philadelphia chromosome. This rearrangement results in the production of chimeric bcr-abl oncoprotein with a constitutive tyrosine kinase activity. Our previous work showed that CML exosomes were involved in modulating angiogenesis, and other work in a different cancer histotype indicated that hypoxia triggers a pro-angiogenic pathway mediated by the release of exosomes. Because, exosomes are involved in the horizontal transfer of information, through the export of miRNAs, we focus on the role of miRNAs isolated from CML exosomes in angiogenesis. *Material and methods*: Exosomes were collected from LAMA84 conditioned medium by different centrifugation steps. The miRNAs found in exosomes were characterised by miRNA-array and RT-PCR validation. HUVEC were treated with exosomes for 6–24 h, analysed for expression of different genes with RT-PCR and evaluated for protein secretion by ELISA. *Results*: Our preliminary data indicated that CML exosomes shuttle miRNAs involved in angiogenesis, we focused on miRNA 22, 126, 150 and 886. We also observed that the treatment of endothelial cells with CML-exosomes down-regulate CXCL12 gene expression, and we are currently investigating whether miRNA 126 and 886, targeting CXCL12, are involved in this process. *Conclusion*: CML-derived exosomes contain miRNAs that can target genes involved in angiogenesis.