Session 15: Immunometabolism and extracellular vesicles in health and disease

Lectures

L.15.1

Immunometabolism in control of immune responses during sepsis

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Immunometabolism is a term coined to describe the changes that occur in intracellular metabolic pathways in immune cells during activation. Pro-inflammatory signals will induce a metabolic switch in leukocytes resulting in up-regulation of aerobic glycolysis and this metabolic change will determine their effector responses. On the other hand, the metabolic state of the organism will impact functioning of leukocytes. Having this in mind, we studied the impact of the inflammatory conditions and/or metabolic state of mice on the course of systemic inflammation to verify leukocyte functioning with emphasis on neutrophils. The latter cells when activated form neutrophil extracellular traps (NETs) which are beneficial during early infection cause bystander damage as sepsis progresses. We verified impact of inflammatory insult on neutrophils and NETs with the Seahorse XF Analyzer and directly in blood vessels with intravital microscopy (IVM) in cells of healthy and septic mice but also animals with obesity. As the field of immunometabolism also demonstrated that metabolites can lead double lives as immunomodulators, we studied impact of itaconate on neutrophils and NETs. Itaconate is a metabolite produced in large quantities in LPS-activated macrophages during a broken Krebs cycle. We showed that itaconate selectively inhibits NET formation and revealed molecules involved in its mechanism (Nrf2, HO-1, Hif-1alpha).

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L.15.2

Towards understanding the role of glycosylation in melanoma-derived extracellular vesicles

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There is growing interest in the role of extracellular vesicles (EVs) as regulators of disease progression. Regarding tumor-derived EVs, they can participate in the formation of a premetastatic niche and promote tumor progression, metastasis, immunosuppression and multidrug resistance. The effect exerted by EVs on recipient cells depends on the molecular composition of their cargo (proteins, lipids, nucleic acids, etc.). The exact mechanisms of sorting bioactive molecules into EVs and the factors affecting the efficiency of this process and EV incorporation into recipient cells are not yet well understood, but there is evidence that glycosylation can modulate not only sorting process and incorporation of EVs, but also EV organ tropism.
In our study, we showed that the microvesicles released by melanoma cells, although originating from specific regions of the cell membrane, contain a unique glycan composition. Moreover, the glycosylation status of the parental cells was responsible for the effect exerted by EVs on the proliferation and migration of recipient cells. In addition, changes in glycosylation resulted in changes in molecular composition of EV as shown by Fourier-Transform InfraRed (FT-IR) spectroscopy and Liquid Chromatography and Tandem Mass Spectrometry (LC–MS/MS).

Oral presentations

O.15.1

Leukemic extracellular vesicles drive immunosuppressive T cell-mediated microenvironment and progression of myeloid leukemia

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Interactions between leukemic and immune cells and their role in leukemia development and resistance are still not clear. This includes immunosuppression which in leukemias remain unexplored and immune checkpoint therapies are not effective. Extracellular vesicles have been implicated as effective drivers of cross-talk between distant cells. Here, we studied the function of CML and AML-derived EVs in remodeling of immunosuppressive effector regulatory T cells (Tregs) and exhausted T cells as well as leukemia progression. We found that Rab27a-dependent leukemic EVs constitute a novel factor that drives immunosuppressive microenvironment by T cells remodeling, associated with progression of myeloid leukemias. This includes EVs-driven specific immunosuppressive subsets of Tregs and promotion of subsets of CD39+ exhausted/dysfunctional T cells leading to adenosine-dependent metabolic immunosuppressive microenvironment. Using spectral flow cytometry we identified two distinct, effector Treg (eTreg) subsets promoted by leukemic EVs. Importantly, vesicle-driven dysfunctional CD39+ T cells were distinct from PD-1+ exhausted T cells. They were present in leukemic patients, showed decreased secretion of IL-6, TNFa, and IFNg and correlated with disease burden. Finally, *in vivo* model of CML revealed that leukemic EVs contribute to elevated Tregs, increased expression of immunosuppressive CD39 and leukemia engraftment, pinpointing role of EVs in immunosuppression and leukemia progression.

O.15.2

The comparison of extracellular vesicles with matrix vesicles derived from human vascular smooth muscle and bone cells

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Aging is associated with a decrease in physiological bone mineralization (BM), but also with an increase in pathological vascular calcification (VC). Vascular smooth muscle cells (VSMCs), the key cell type involved in VC, display features of bone cells. Calcifying VSMCs secrete extracellular vesicles (EVs), which are similar to matrix vesicles (MVs) derived from mineralizing bone cells. Both types of vesicles show translational potential for intercellular communication. Due to their variable diameter and composition, conventional techniques are inadequate for vesicle counting and phenotyping. Many aspects of their biogenesis, transport and involvement in hydroxyapatite (HA) formation remain unanswered.
Our major goal was to understand the pathological mechanism leading to trans-differentiation of VSMCs into mineralization-competent cells during VC and to compare it with the physiological mechanism of HA nucleation during BM. Special attention was given to two families of proteins: tissue-nonspecific alkaline phosphatase (TNAP) and calcium- and phospholipid-binding annexin A6 (AnxA6) in human cell lines: vascular HCASMC versus bone Saos-2 cells. We have determined biomarkers of mineralization using different techniques: NanoSight vesicle tracking, TEM nanogold visualization, FTIR mineral analysis and X-ray ion mapping. We conclude that the comparison of EVs with MVs may serve as a possible diagnostic tool to monitor the progression of cardiovascular versus skeletal diseases.

O.15.3

Single vesicle imaging flow cytometry approach to unravel the molecular profile of endometriosis-related extracellular vesicles as a source of potential biomarkers

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Endometriosis is a chronic gynaecological disorder with no effective treatments or biomarkers for early diagnosis, despite its high prevalence. Since extracellular vesicles (EVs) are present in almost all body fluids, they have been recently investigated as easy accessible source of biomarkers in liquid biopsy. Here, EVs were isolated from plasma and peritoneal fluid (PF) of endometriosis and control patients using SEC and were verified by WB, fluorescent mode NTA, and imaging flow cytometry (IFCM). The molecular phenotype of EVs was determined by multiparametric IFCM using a specially designed panel of antibodies, which included detection of antigens that are typically elevated during chronic inflammatory states, or are associated with the development of early endometriotic lesions, and immune suppression. We detected a heterogenous collection of EVs in plasma and PF samples from endometriosis patients and controls. These vesicles exhibit general features associated with small EVs, and contained bona fide EV markers. Upon performing single EV analyses on the IFCM platform, we learned that endometriosis-derived EV populations contain a wide range of molecules and at least some of them are associated to endometriosis pathogenesis. Our results, imply endometriosis-specific small EV signatures in the patients' plasma. After evaluation, these EV signatures may serve as potential non-invasive diagnostic biomarkers or therapeutic targets in endometriosis in the future.

Posters

P.15.1

Markers of toxicity and cell-cell communication through extracellular vesicles in primary astrocytes exposed to polystyrene nanoparticles

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The continuous increase in plastic production has triggered a serious environmental crisis. Under physical forces, plastic waste disintegrates into nano- and microparticles (PS-NPs and PS-MPs). PS-NPs exhibit high reactivity within biological systems and induce toxic effects. The effects of nanoplastic exposure on cellular functions remain unclear and require detailed research. In this study, we investigated the impact of 25 nm PS-NPs on primary astrocyte cultures. Primary astrocytes were cultured in complete growth medium for viability assays, immunocytochemistry and preparation of extracellular vesicles (EVs). The cultures were exposed to PS-NPs in concentration range of 0.5 - 50 μg/mL at different times. Fluorescence microscopy revealed that PS-NPs were internalized by astrocytes and caused time- and dose-dependent decrease in cell viability as measured by MTT and LDH assays. Furthermore, we observed increased fluorescence in EVs isolated from astrocytes exposed to high concentrations of fluorescent PS-NPs which indicates incorporation of NPs into the bioactive cargo of EVs and their possible transfer between cells. The results show that PS-NPs enter the cell and exert cytotoxic effect in primary astrocytes. Intercellular communication system via EVs may be involved in the mechanism of nanoplastic toxicity. However, more research is needed to understand molecular mechanisms.

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P.15.2

Autophagy inhibition promotes increased exosomes secretion in vascular smooth muscle cells, what imitates senescence phenotype.

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Senescence is a state of irreversible growth arrest that can be induced by various stressors. Exosomes derived from senescent cells have been found to promote senescence in neighboring cells, thus propagating the senescent phenotype. Furthermore, specific cargo molecules, packaged within exosomes can modulate senescence-associated secretory phenotype (SASP) and influence the pro- or anti-senescent signaling pathways. Thus the aim of our study was to analyze EVs secreted by human vascular smooth muscle cells (VSMCs) at early and late stage of senescence. We used the model of VSMCs induced to senescence by doxorubicin treatment and analyzed different senescence markers after 1 and 4 weeks upon senescence induction. Our studies revealed that the level of expression of majority of senescence markers decrease in a time-dependent manner although the number of senescent cells remained constant. Moreover development of senescence phenotype correlated with autophagy inhibition. In contrary, we observed increased level of multivesicular bodies (MV), which participate in exosomes formation and secretion, in late senescent VSMCs. In addition, we have shown that late senescent cells secrete more EVs than young or early senescent VSMCs Moreover, we can modulate the number of secreted EVs by treating VSMCs with inhibitors of autophagy or mTOR such as BafA1 or rapamycin. This result indicates that disturbances of autophagy facilitate upregulation of EVs secretion by senescent VSMCs.

P.15.3

Role of plasma – derived small extracellular vesicles (sEVs) in the pathogenesis of chronic rhinosinusitis

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Introduction: Chronic rhinosinusitis (CRS) is disease affecting almost 5-12% of human population. It occurs in two main subtypes: without nasal polyps (CRSsNP) and with nasal polyps (CRSwNP). Molecular background of disease remains still elusive. CRS associates with chronic inflammation, damage of upper airway epithelium, tissue remodeling and altered immune response. Currently, small extracellular vesicles (sEVs) are considered as the contributors to CRS development or possible agents to use in the diagnostic or therapeutic approaches. The aim of our study was to characterize the plasma – sEVs isolated from patients with CRSsNP or CRSwNP and control group.
Methods: sEVs were isolated with filtration, centrifugation and Size Exclusion Chromatography (SEC), measured with Nanoparticle Tracking Analysis (NTA), Western Blot (WB) and Cryo-Electron Microscopy (Cryo-EM).
Results: NTA indicated the sEVs size range from 83 to 109 nm and concentration from 1,90E+09 to 2,6E+11 particles/mL and differences in the concentration value between patients. WB claimed positive expression of CD9, CD63 tetraspanis and negative of Grp94. Cryo-EM pointed out – characteristic shape, membrane bilayer and size comparable with NTA.
Conclusion: In our study, we claimed with the SEC isolation method the presence of sEVs in the plasma, however evaluation of their cargo and function need further investigation.

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P.15.4

The effect of perinatal fluoride exposure on morphine-related neuroinflammation

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Introduction: Morphine shows immunomodulatory and proinflammatory properties mediated by enhancing neuronal excitability activation of microglia and CNS astrocytes. Also, fluoride exposure is associated with neurotoxicity by inducing oxidative stress and apoptosis of neurons.
Aim: To evaluate if pre-exposure to morphine changes the response to morphine administration in terms of neuroinflammation.
Materials and methods: In the striatum, cerebellum, prefrontal cortex, and hippocampus of rats from 4 studied groups (control, morphine-exposed, fluoride-exposed, morphine+fluoride-exposed) the expression of COX-1, COX-2, Iba1, GFAP was estimated (RT-PCR, Western blot, IHC)
Results and conclusions: We proved that both morphine administration and fluoride exposure influences inflammatory response by changing the expression of COX-1, COX-2, Iba1, and GFAP in structures associated with dependence development (prefrontal cortex, striatum, hippocampus, cerebellum). We have demonstrated that both COX-1 and COX-2 expression in morphine-dependent rats is affected by fluoride pre-exposure, and the changes are structure-dependent. Also, we showed the active astrogliosis process (increased GFAP expression) in all structures of morphine-dependent rats, a structure-dependent effect of morphine on Iba1 expression, and indicated that fluoride pre-exposure may influence this microglia activation.