Session 4: Metabolism and Omics

Lectures

L.04.1

An evidence that SARS-Cov-2/ COVID-19 spike protein (SP) damages hematopoietic stem/progenitor cells in the mechanism of pyroptosis in Nlrp3 inflammasome-dependent manner

Magdalena Kucia

Department of Regenerative Medicine Center for Preclinical Research (CePT) of the Medical University of Warsaw, Poland
Magdalena Kucia <magdalena.kucia@wum.edu.pl>

Mounting evidence accumulates that hematopoietic stem/progenitor cells and endothelial progenitor cells are damaged during severe SARS-CoV-2 infection. It has been reported that patient infected with COVID-19 are frequently presented with anemia, lymphopenia, and thrombocytopenia. It is known that virus may enter cells and, directly in case of productive infection, lead to their irreversible damage. We have proposed that interaction of SP with the target cell surface receptors induces intracellular hyperactivation of Nlrp3 inflammasome which may lead to cell death by pyroptosis. The adverse effects of infection on stem cell compartments result from the uncontrolled hyperactivation of the Nlrp3 inflammasome. We noticed that exposure of human UCB-purified HSCs to recombinant SP led to upregulation of expression for Nlrp3 inflammasome. We detected elevated levels of IL-1β in the conditioned media from cells exposed to SP. Release from cells of IL-1β in a caspase-1-dependent manner is an important indicator of Nlrp3 inflammasome activation. We have analyzed the proteome of HSPCs-derived CM after exposure to recombinant SP. We observed that exposure to SP upregulates the expression of proteins involved in the positive stimulation of the immune system, TLR4 pathway, and proteins that positively impact apoptosis, necrosis, and pyroptosis. We detected decrease in expression of proteins involved in the positive regulation of cell proliferation and differentiation.

L.04.2

Role of extracellular vesicles in cellular crosstalk in cancer and inflammatory conditions

Małgorzata Czystowska-Kuźmicz

Department of Biochemistry, Medical University of Warsaw, Poland
Małgorzata Czystowska-Kuźmicz <mczystowska@wum.edu.pl>

Extracellular vesicles (EVs), heterogenous nano-sized double-membrane vesicles secreted by cells, have been identified as key messengers of intracellular communication in physiological and pathological conditions. By transferring a rich molecular cargo, EVs can shape the molecular pathways and metabolism of different recipient cells.
In cancer, tumor-derived EVs are increasingly acknowledged as a sort of the so called "liquid tumor biopsy", since their molecular and genetic signatures resemble those of their parent cancer cells. In addition, it has been shown that that they play a key role in driving cancer progression, interfering with anti-tumor responses, propagating inflammatory pathways and reprogramming the tumor microenvironment into a cancer-promoting milieu.
Furthermore, the molecular signature of EVs present in patients' biofluids, connected to immune-suppressive and inflammatory responses, may be a non-invasive biomarker of the immune dysfunctions and significantly affect therapy in several diseases, including cancer.
Finally, apart from the pathological nature of EVs, they are also increasingly used for anti-inflammatory therapy, like stem-cell-derived EVs. Recently evidence has accumulated that the therapeutical effect of these cells depends mainly on the paracrine action of secreted EVs. There is a trend to use stem-cell-derived EVs as replacement for cells in future clinical treatments to avoid the safety concerns associated with live cell therapy.

L.04.3

Tools for real-time metabolism analysis in live cells and for exploring heterogeneity of samples with single cell transcriptome resolution

Joanna Zimniak

Altium International Sp. z o.o. Warszawa, Poland
Joanna Zimniak <jzimniak@perlan.com.pl>

Metabolism is a key feature and critical indicator across a range of research areas. Agilent Seahorse XF metabolic analyzers and test kits make cellular bioenergetic studies simple, efficient and user-friendly. Agilent Seahorse XF metabolic analyzers are the most cited and most used technology platform for measuring cell metabolism in live cells in real time. XF analyzers are the only commercially available instruments for scientific research that simultaneously measure the two major energy producing pathways of the cell – mitochondrial respiration and glycolysis – in living cells, in a microplate, in real time and does not require the addition of dyes, labels, or reporters. Researchers are utilizing Agilent Seahorse XF technology for faster, better, and more accurate measurements of cellular metabolism. ScaleBio technology for single cell sequencing unlocks detailed information about the complexity of cells and has transformed our understanding of cellular biology. Many single-cell phenotyping applications rely on surveying many cells in a population to get a full picture of the cellular architecture in a tissue or disease model. With combinatorial indexing technology from ScaleBio, single-cell studies can be massively scaled to accomodate larger projects, expanding our knowledge of cell phenotypes in many areas of research. This instrument-free workflow only requires reagent kits, basic laboratory equipment, and simple plastics.

Posters

P.04.1

LC-MS/MS profiling of *Lactococcus* and *Leuconostoc* excretome: vitamins B, short-chain fatty acids and lactic acid in culture media

Mariusz Radkiewicz[1], Emilia Samborowska[1], Magdalena Kowalczyk[2], Julia Kopczyńska[2], Olha Kostiuchenko[2]

[1]Mass Spectrometry Laboratory, Institute of Biochemistry and Biophysics Polish Academy of Science, Warsaw, Poland; [2]Laboratory of Lactic Acid Bacteria Biotechnology, Institute of Biochemistry and Biophysics Polish Academy of Science, Warsaw, Poland
Mariusz Radkiewicz <mar.radkiewicz@gmail.com>

Properties and composition of the intestinal microbiome becomes an emerging issue regarding many illnesses. Certain lactic acid bacteria (LAB) strains, mostly belonging to lactobacilli are already marketed as health supplements. They can engage in regulating imbalanced gut microbiota, stimulating the immune system and dietary intake in various diseases related to general dysbiosis. Our project aims to identify new potential probiotics among other LAB *Lactococcus* and *Leuconostoc* strains with exquisite yield of nutrients excretion.

Metabolic analysis of 100 media samples, divided to groups consisting of varying pre-selected strains grown on different selective media, was performed using LC-MS/MS analysis. Profiles consisting of 12 vitamins B, 10 SCFAs and lactic acid were quantified. Derivatisation of SCFAs and lactic acid was applied in order to upgrade their chromatographic properties and increase method sensitivity.

Several strains were found to produce high numbers of vitamins B, SCFAs and lactic acid. All lactococcal strains produced B6 at high amounts (reaching 2.13 μg/mL), two strains – B9 (average 1.08 μg/mL) and one – B7. The main vitamins produced in higher amounts by leuconostocs are B2 and B3. All strains excreted high yield of lactic acid and acetic acid. Our study indicated strains producing pro-health metabolites which can be potentially used as a probiotics for patients with vitamins B deficiency or gut dysbiosis.

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