Abstracts

Flow cytometry in the study of immune system involvement of neurodegenerative disorders

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Neurodegenerative disorders, especially the most common including notably the Alzheimer's disease (AD), are for many years a field of intensive research, aiming at understanding their pathomechanism. Mostly however, this research is targeted at the central nervous system deterioration itself. In recent decade, there is rising awareness among specialists that AD pathomechanism is in fact involving impaired (increased) peripheral immune and inflammatory reaction to pathological proteins (beta-amyloid and phospho-tau). Flow cytometry, including advanced analysis of cellular proliferation dynamics (DCT) that we had developed, allowed for deeper insight into specific immunity of AD patients. We have demonstrated that in addition to known, mildly intense stimulation of below 1% peripheral T cells, beta-amyloid exerts a nonspecific immunomodulatory effect, significantly increasing both proliferation and proinflammatory cytokine secretion by the lymphocytes stimulated by other (mitogenic and possibly antigenic) agents. This results in a population shift among peripheral blood T cells towards an accumulation of terminal memory (TEMRA) cells, resembling that occurring during otherwise healthy ageing. Detailed molecular mechanisms behind these phenomena require further studies.

CyTOF technology: from the bench to data management

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The cytometry by time of flight (CyTOF) combines the flow cytometry to the mass spectrometry to reach an unprecedented number of parameters analysed at the single cell level. By the use of this new technology, it is possible to analyse till 100 parameters on a single cell. Importantly, the use of mass spectrometry, add several advantages like the absence of spectral overlap and an absolute quantitation. The analysis of these high-multiparameters dataset requires the use of new software solutions. In particular, unsupervised methods will be the most appropriate to this kind of new dataset. A database approach is also required to keep track of this large amount of data and to correlate CyTOF datasets to all the other data generated in the laboratory.

Flow cytometry analysis of cell cycle disturbances during senescence of cancer cells

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Cellular senescence of cells is characterized by permanent growth arrest. Cells remain alive but they are refractory to mitogen stimulation. According to literature those cells do not pass restriction point R1 and exit cell cycle during G1 phase. However our own experiments as well as data coming from literature shows that cellular senescence could be connected with aberrant cell cycle. Using flow cytometry analysis of DNA content of cancer cells induced to senescence by doxorubicin treatment, we revealed that those cells undergo polyploidyzation via endoreduplication. Senescent cells were arrested in polyploid G1 phase of cell cycle since they did not incorporate BrdU and expressed high level of G1 cyclin D1. Moreover we showed that aberrant cell cycle could be also a primary reason for senescence induction in cancer cells. Flow cytometry analysis of cells arrested in mitosis due to curcumin treatment as well as measurement of the level of SA-β-galactosidase activity enable to correlate mitosis disturbances with senescence induction. The fate of polyploid senescent cells must be carefully studied due to the risk of induction of genomic instability upon cancer treatment.

Signal monitoring in leukemic cells and flow cytometry

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A technology that allow us to track the cell signaling alterations at the individual cell level, represents a terrific advance to determine phenotypes of individual cancer cells. Using intracellular flow cytometry, it is possible to do it (Irish JM et al. Cell 2004). Our first goal has been to validate this technology « in situ » using commercially available antibodies against the activated forms of the PI3K effector, the serine/threonine kinase, Akt in normal cells such as primary human T lymphocytes (activated or not with CD3 plus CD28 antibodies). Then we improved this method to apply this detection of cell signaling events to primary cancer cells (Firaguay G and Nunès JA. Science Signaling 2009). We are currently identifying cell signaling signatures in acute myeloid leukaemia patients.

Flow cytometry in the studies of prosurvival mechanisms in leukemia cells

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Chronic myeloid leukemia is a disease of hematopoietic stem cell. CML cells in the blast crisis stage of the disease as well as CML stem cells are highly resistant to therapy and activate different prosurvival mechanisms. Recently we have discovered a novel prosurvival pathway activated in CML cells, involved in the development of the resistance to imatinib. In our studies, we used different flow cytometry methods to investigate apoptosis and cell death of CML cells, proliferation and cell cycle. Moreover, we studied different parameters of the cell-cell interactions detected *in vivo* in the co-culture of leukemia and stroma cells. To this end we developed protocols to distinguish different types of co-cultured cells by cell tracking and simultaneously analyze their survival and proliferation rate by fluorescent dyes together with detection of the level of intracellular proteins using multicolor flow cytometry.

Workshop on scientific publication and reviewing process in Cytometry Part A and the special aspects in publishing the Cytometry data

Attila Tarnok, University of Leipzig, Germany; the Editor-in-chief of the *Cytometry Part A* Journal

This will be dedicated to publishing process in Cytometry Part A and good standards to publish high quality data in cytometry. In general, the Session will be divided into three parts and dedicated to three different issuess: 1) How to write a good manuscript; 2) Reviewing and manuscript processing and 3) Flow cytometry data presentation guidelines, including information about the MIFlowCyt: Minimum Information about a Flow Cytometry Experiment – recommended for Cytometry A publications since October 2010. Each of the parts will give a detailed information and possibility to discuss some unclear aspects of the above subjects.