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Evaluation multiple intracellular phospho-signalling events as biomarker on single cell level using flow cytometry

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Research of cell signalling pathways, networks, and interactions that cells require to function is a major scientific challenge. Kinases and Signal Transducers and Activator of Transcription (STAT) proteins regulate many aspects of cell growth, survival and differentiation. The transcription factors of this family are activated by kinases (e.g. JAK) and dysregulation of this pathway is frequently observed in primary tumors and leads to increased angiogenesis, enhanced survival of tumors and immunosuppression. Usually, cell signalling analysis has been performed using Western blots on large cell populations. If performed on a complex combination of cells, such as peripheral blood, this technique would not yield signalling information of individual cellular subsets within the whole population. Intracellular flow cytometric analysis of protein phosphorylation has emerged as a powerful tool in the field of immunological signalling, which allows to analyze quickly and accurately the cellular subsets in heterogenous populations. Here we are describing the evaluation of multiparametric flow cytometry application to investigate the early phosphorylation events of STAT3, STAT5 and STAT6 in human peripheral blood. For this purpose we evaluated the brief application of cytokines and mitogens *in vitro* and analyzed the phosphorylation of these STAT proteins in the human leukemic monocyte lymphoma cell line U937. Individual cytokine combination (IL2, IL4, IL6 and GM-CSF) of treatment were assessed to ensure the detection of specific STAT phosphorylation. In conclusion, detection of STAT3, STAT5 and STAT6 phosphorylation through intracellular staining by Flow Cytometry in multiparametric analysis enables the study of cell signaling events in an approach that is quick, sensitive and quantitative on the single cellular level compared to conventional methods.

Biography

Raif Yucecel is the Head of the Iain Fraser Cytometry Centre at the University of Aberdeen, servicing and consulting in biology, biochemistry, and medical research division labs on Cytometry, and also on Small Animal Imaging Applications. His research is mainly focused on different application possibilities of Cytometry, such as the investigation of cellular signalling in human blood, stem cell analysis, cancer research, biomarker discovery, immunology, clinical research, microbiology cytometry, marine and ocean biology. As Principal Investigator he is also actively involved in teaching and consulting on Cytometry. Dr. Raif Yucecel is a member of various Cytometry and Imaging society (ISAC, FCUK, DGfZ, WMIS, ISMRM).

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