THE ACTIVATION PROFILING OF STAT IN HUMAN WHOLE BLOOD SAMPLES BY FLOW CYTOMETRY WITH AN APPLICATION FOR HUMAN CLINICAL TRIALS

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ABSTRACT

Background: The JAK-STAT signaling pathway is responsible for transmitting information from the cellular cytoplasm to the nucleus, modulating DNA transcription and expression of genes involved in immunity, proliferation and oncogenesis. In the development and progression of certain types of cancers, involvement of a combination of different STAT proteins (STAT1, STAT3 and STAT5) are usually involved. Flow cytometry, a high complexity testing, has become more and more usefully in studying certain pathways due to the development of antibodies and kits that measure the reactions.

Methods: Experiments were conducted with a cell line and human whole blood samples activated for STAT expression. Cells were processed using the Millipore FlowCellect STAT Activation kit with pSTAT1, pSTAT3 and pSTAT5 A/B. Cells were acquired on the BD FACSCanto™II flow cytometer.

Results: Results showed an increase in the STAT signals upon activation.

Conclusion: The profiling of STAT activation can be evaluated using Flow Cytometry over a course of time. Following their activation over time can be very advantageous for researcher in clinical trials. There can be great understanding of the processes that happen when the cells replicate and transform nonstop

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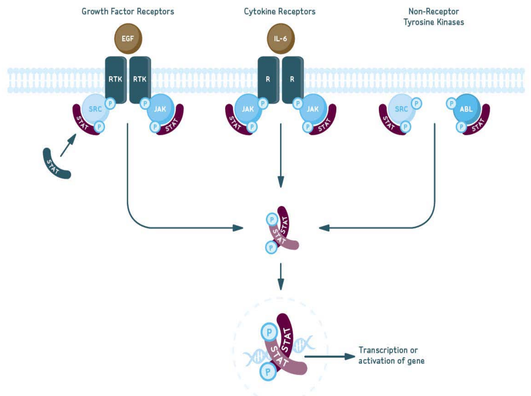
INTRODUCTION

The Signal transducers and activators of transcription (STAT) comprise a family of cytoplasmic transcription factors that transmit signals, usually generated at cell surface receptors, to the nucleus where STATs bind to specific DNA promoter sequences and thereby regulate gene expression. Compared with normal cells and tissues, constitutively activated STATs have been detected in a wide variety of human cancer cell lines and primary tumors. STATs are activated by tyrosine phosphorylation , which is normally a transient and tightly regulated process. Persistent signaling of specific STATs has been demonstrated to directly contribute to oncogenesis by stimulating cell proliferation and preventing apoptosis.



http://www.millipore.com

MATERIALS AND METHODS

Modification: Due to the unavailability of human whole blood and Millipore FlowCellect STAT Activation kit, experiments were conducted on BV-173 cells and a method was developed using individual components from BD including Cytofix buffer, BD Perm Buffer and BD pSTAT1 AF647, pSTAT3 PE, pSTAT5 PECy7.

Methods: BV-173 (B Cell leukemia derived cell line) were stimulated with 125 ng of IFN-γ, 25 ng of GM-CSF and 50ng of IL-6 for 9-11 min at 37°C. Cells were centrifuged, washed and fixed at 2-8°C. Cells were washed with Stain Buffer (BSA) and permeabilized on ice. Cells were centrifuged, washed and stained with pSTAT3 PE, pSTAT5 PECy7, pSTAT1 AF647. Cells were washed with Stain Buffer (BSA) and acquired on the BD FACSCantoII flow cytometer with BD FACSDiva software.

RESULTS

Unstimulated Cells | Stimulated Cells

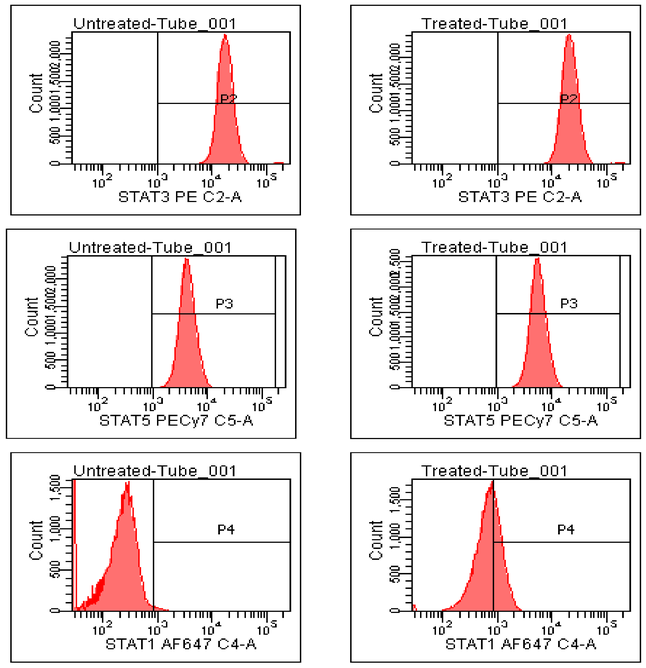
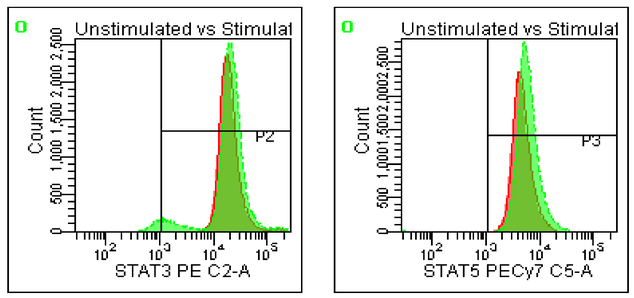


Figure 1: BV-173 cells were unstimulated (left) and stimulated (Right) with 125 ng of IFN-γ, 25 ng of GM-CSF and 50 ng of IL-6 for 9-11 min at 37°C. Cells were fixed, permeabilized and stained with pSTAT3 PE, pSTAT5 PECy7, pSTAT1 AF647

	%Parent (Untreated)	% Parent (Treated)
P2	99.8	99.7
P3	99.7	99.8
P4	1.6	32.0

UnStimulated vs Stimulated



RESULTS (cont.)

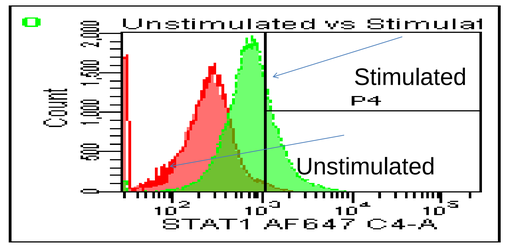


Figure 2 represents an overlay of unstimulated (left) and stimulated (Right) cells. Cells were stimulated with 125 ng of IFN-γ, 25 ng of GM-CSF and 50 ng of IL-6 for 9-11 min at 37°C. Cells were fixed, permeabilized and stained with pSTAT3 PE, pSTAT5 PECy7, pSTAT1 AF647

CONCLUSIONS

•The Profiling of STAT activation can be evaluated using Flow Cytometry over a course of time. It can be advantageous for researcher in clinical trials.

•BV-173 cells show an increase in pSTAT1 signal upon activation in stimulated cells.

•BV-173 cells show pSTAT3 activation in both unstimulated and stimulated cells.

•BV-173 cells show pSTAT5 activation in both unstimulated and stimulated cells.

•In the future, we will be doing this assay in human whole blood and other cell lines.

REFERENCES

a. FlowCellect Multi-STAT Activation Profiling kit. 2016. Technical Data Sheets. http://www.millipore.com

b. Ralf Buettner,, Linda B. Mora, and Richard Jove. Activated STAT Signaling in Human Tumors Provides Novel Molecular Targets for Therapeutic Intervention. Clinical Cancer Research, 8: 945-954,2002.

ACKNOWLEDGEMENTS

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