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The presence or absence of IL-3 during long-term culture of Flt3-ITD and c-Kit-D816V expressing Ba/F3 cells influences signaling outcome

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The murine lymphoid cell line Ba/F3 is widely used as model system for studies on hematopoietic cell signaling [1-9]. Since its dependency of IL-3 can be rescued by several receptor tyrosine kinases, Ba/F3 cells are a good model to study the signal transduction and biological response of receptor tyrosine kinases such as c-Kit and Flt3. The most commonly occurring mutation in c-Kit, D816V, and the Flt3 internal tandem duplication (ITD) mutation renders Ba/F3 cells independent of IL-3 for survival and Ba/F3 cells transduced with the oncogenic mutants of c-Kit and Flt3 induce tumors in mice. In previously published studies on transfected Ba/F3 cells, different culture conditions have been used for the transfected cells. For example Arora *et al* and Kazi *et al* used medium supplemented with IL-3 [1, 2], while Leischner *et al* and Zirm *et al* used medium lacking IL-3 for culture of Ba/F3-Flt3-ITD cells [3, 4]. Similar discrepancies were found in paper describing studies on Ba/F3-c-Kit-D816V cells with some investigators using medium with [5, 6] and some investigators using medium without IL-3 [7, 8] for long-term culture. Since there are differences in the signaling pathways induced by IL-3 as compared to the oncogenic mutants of Flt3 and c-Kit that might influence gene expression and ultimately cell behavior, we decided to investigate whether different culture conditions could influence the signal transduction outcome and biological responses of Flt3-ITD and c-Kit-D816V, respectively, in Ba/F3 cells.

To address this question, Ba/F3-Flt3-ITD and Ba/F3-c-Kit-D816V cells were generated by retroviral transduction of constructs in pMSCVpuro vector followed by puromycin selection in the presence of IL-3 in the culture medium. Cells were then further cultured for 1-2 weeks in presence or absence of IL-3 in the culture medium. Immediately preceding the experiments, IL-3 was removed from the medium for a period of 4 hours before the experiment was started. As measured by a cell counter, the cell size of both Ba/F3-Flt3-ITD and Ba/F3-c-Kit-D816V cells significantly decreased upon culture in the absence of IL-3 (Fig. 1A) while the expression of either receptor did not change (Fig. 1B, C). This suggests that signals from IL-3 and the signals from the oncogenic mutants of c-Kit and Flt3 have different impact on cell growth. Upon ligand binding, receptor tyrosine kinases activate downstream signaling cascades. In the absence of IL-3 in the growth medium during culture, tyrosine phosphorylation of either receptor increased both in the presence and the absence ligand stimulation (Fig. 1B, C). Both the PI3K/Akt and the Ras/Erk signaling pathways can be activated by either Flt3 or c-Kit and play important roles in the biological responses mediated by either receptor [10]. Activation of both Akt and Erk was increased when cells were long-term cultured in the absence of IL-3 (Fig.

1D, E, F, G), and under these conditions receptor phosphorylation was also increased. Activation of STAT5 is uniquely occurring in cells expressing Flt3-ITD but not wild-type Flt3. We could demonstrate that also STAT5 phosphorylation was stronger when Ba/F3-Flt3-ITD cells were grown in medium without IL-3 (Fig. 1H). In cells long-term cultivated in the absence of IL-3, the proliferative response and cell survival was stronger than in cells long-term cultivated in the presence of IL-3 (Fig. 1I-L). Furthermore the time dependent growth curves also suggest that IL3 withdrawal potentiates oncogenic Flt3 and c-Kit-induced cell proliferation (Fig. 1M-P). Taken together, these data indicate that culture of this type of cells in the absence or presence of IL-3 in the growth medium influences the signal transduction and biological response mediated by oncogenic mutants of receptor tyrosine kinases c-Kit and Flt3.

We don't know the reason for this difference. It might be a matter of selection for certain cell types during long-term culture. Cells that can survive well and proliferate fast under certain conditions might overgrow the cell culture after long-term culture. Ba/F3-Flt3-ITD and Ba/F3-c-Kit-D816V cells grown in the medium without IL-3 will be selected for those cells that have stronger activation of the receptors and downstream signaling pathways, leading to a selection of cells with stronger oncogenic signaling. Alternatively, the presence or absence of a cytokine such as IL-3 might alter the gene expression pattern and thereby alter the signaling response in cells.

Author contributions

JUK, JS and LR designed and analyzed the research and wrote the paper. JUK and JS performed the research. JUK, JS and LR edited the manuscript.

Conflicts of interest

The authors have no conflicts of interest financial or otherwise to declare.

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Figure legends

Fig. 1. IL-3 withdrawal increases receptor activation, cell proliferation and cell survival. A-H: Cells were washed with PBS for three times and starved for 4 hours followed by stimulation with Flt3 ligand (FL) or stem cell factor (SCF) for 5 minutes. Cells were then lysed and processed for Western blotting with respective antibodies. I and J: Cells were washed with medium for three times and seeded in a 96-well plate with a concentration of 10000 cells per well. Cells were then grown for 48 hours with or with FL/SCF followed by Presto blue cell viability assay. K and L: Cells were washed with medium for three times and seeded in a 12-well plate with a concentration of 100000 cells/ml. Cells were then grown for 48 hours with or with FL/SCF followed by Anexin V/7AAD apoptosis assay. M-P: Cells were washed with medium for three times and seeded in a 12-well plate with a concentration of 80000 cells per well. Cells were then grown for different time points with or with FL/SCF followed by Trypan blue exclusion assay.

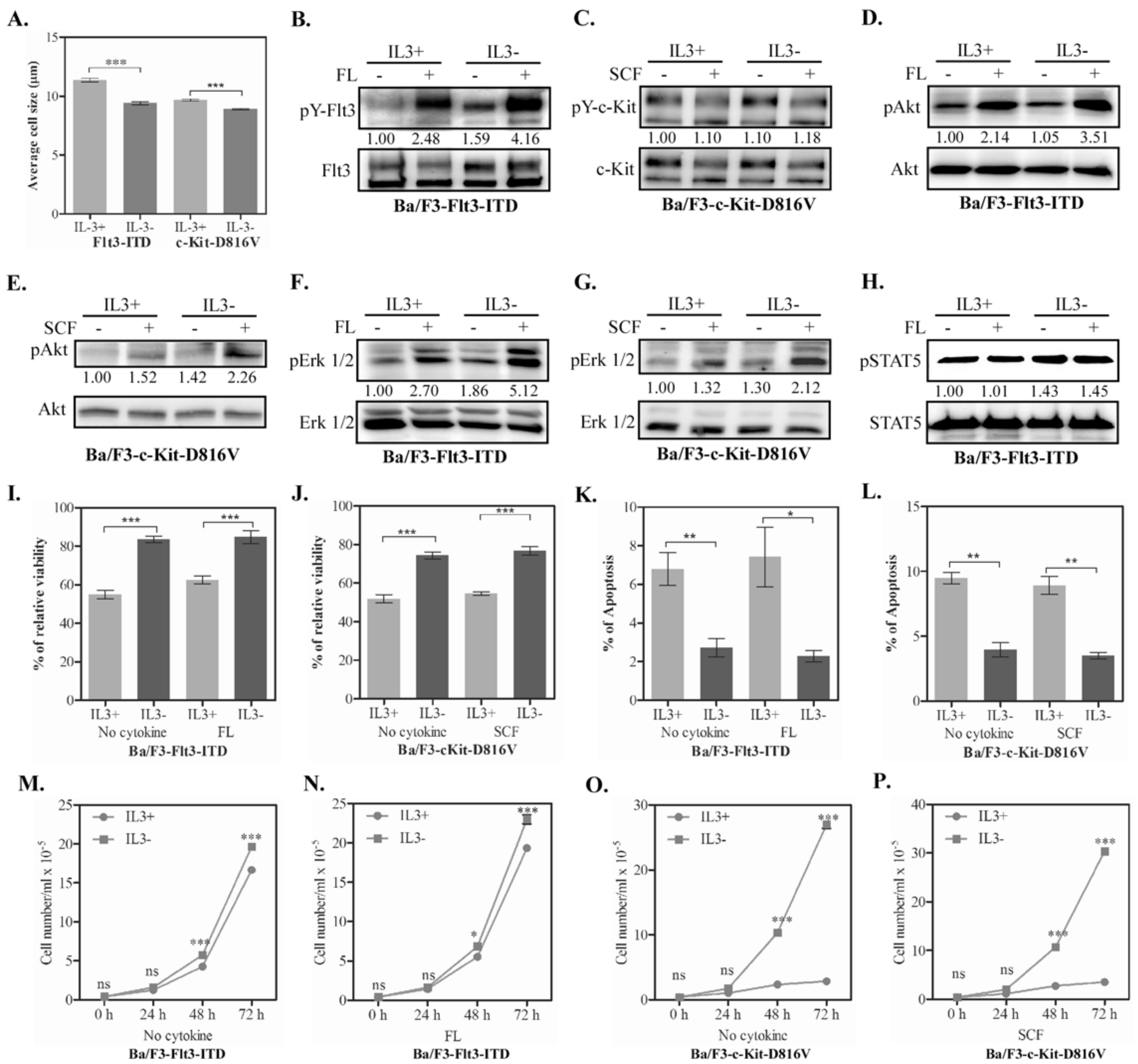

Figure 1