Investigating the Effects of SFK and Mek Inhibitors on G-CSF Signaling

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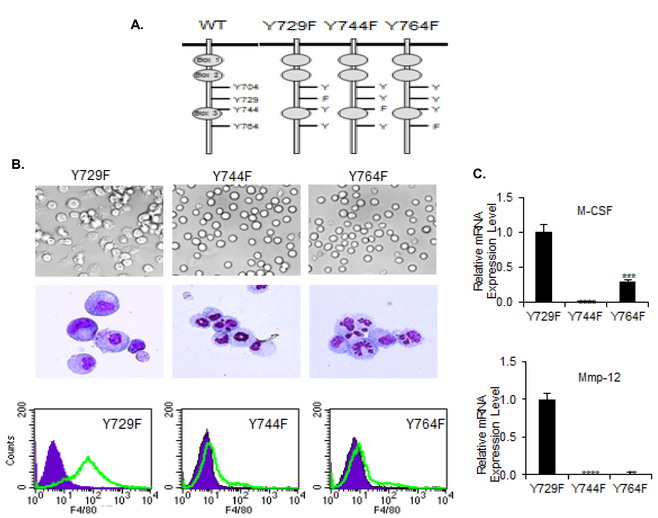
Abstract

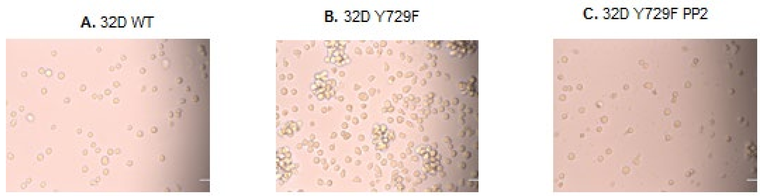
Granulocyte-Colony Stimulating Factor (G-CSF) is known as a growth factor that positively regulates neutrophil development. G-CSF supports neutrophil differentiation by binding to the G-CSF Receptor (G-CSFR), leading to the activation of downstream signaling pathways such as the Jak/Stat, Mek/Erk1/2 and Src-Family Kinases (SFKs). However, the molecular mechanisms by which G-CSF acts in myeloid development remain incompletely understood. The SFKs have been shown to play a critical role in monocyte development, but significantly less is known about their roles in neutrophil development. The Dong lab recently showed that tyrosine (Y) 729 in the cytoplasmic domain of G-CSFR controls neutrophil versus monocyte development of myeloid precursors by regulating the Mek /Erk1/2 pathway and the downstream transcription factors c-Fos/Egr-1. As the SFKs have been shown to activate the Mek/Erk1/2 signaling pathway, we examined whether they are involved in the regulation of neutrophil development in response to G-CSF. We show here that SFKs appeared to regulate the different aspects of neutrophil versus monocyte development. Additional studies are needed to further clarify the roles of SFKs in myeloid development.

Figure 1: Tyrosine (Y) 729 of G-CSFR Is Required for Neutrophil Development in Response to G-CSF



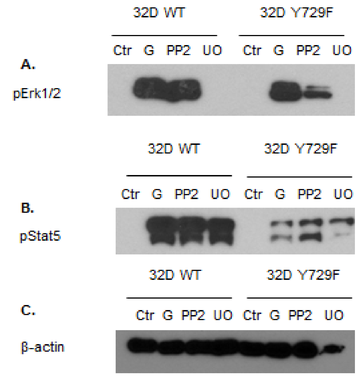
(A) Schematic representation of G-CSFR and its mutants. 32D cells transfected with the different G-CSFR forms were cultured in G-CSF for 4 days. **(B)** Cell growth behaviors, morphology and surface expression of the monocyte differentiation marker F4/80 were examined . **(C)** The expression of monocyte differentiation markers M-CSF and Mmp-12 was examined by real-time RT-PCR. The data were graciously provided by Dr. Fan Dong.

Figure 2: Effect of SFK Inhibitor PP2 on Cell Attachment in 32D/Y729F cells



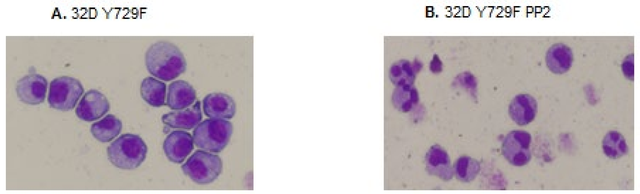
(A-C) 32D cells expressing the indicated G-CSFR forms were cultured over a 3 day period with G-CSF without or with PP2 prior to examination of cell attachment.

Figure 3: Effects of PP2 and UO126 on G-CSF-Stimulated Erk1/2 and Stat5 Activation



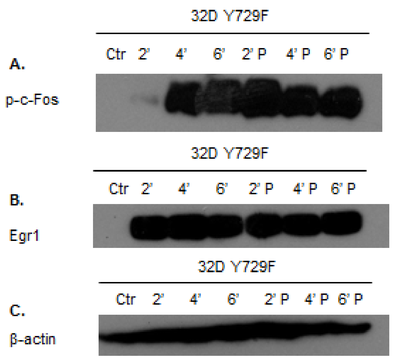
(A-C) 32D WT cells and 32D Y729F cells were utilized in this experiment. In the control (Ctr) samples the cells were left untreated while G samples were only treated with G-CSF. PP2 samples were treated with PP2 along with G-CSF whereas UO samples were treated with both UO126 and G-CSF. Erk1/2 and Stat5 phosphorylation were both examined by Western Blot analysis using β-actin as a sample loading control.

Figure 4: Effect of PP2 on Cell Morphology Changes In Response to G-CSF



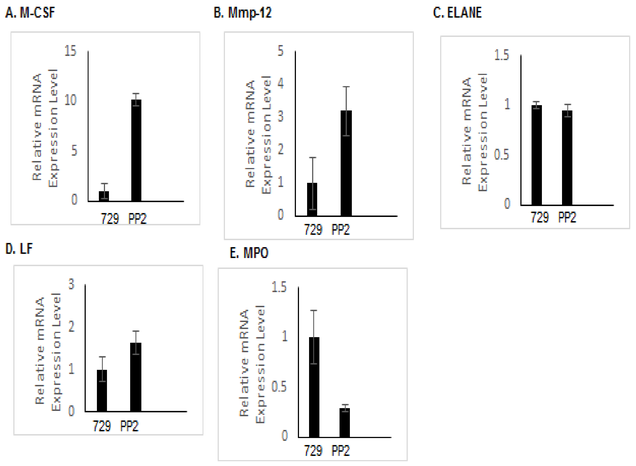
(A-B) 32D Y729F cells were utilized in this experiment. One set of 32D Y729F cells were cultured in G-CSF for 7 days. Another set of 32D Y729F cells were cultured in both G-CSF and PP2 for 7 days. Giemsa May-Grunwald staining and 40x magnification lenses were used to visualize the cellular morphology.

Figure 5: Effects of PP2 on G-CSF-Induced Expression of p-c-Fos and Egr-1



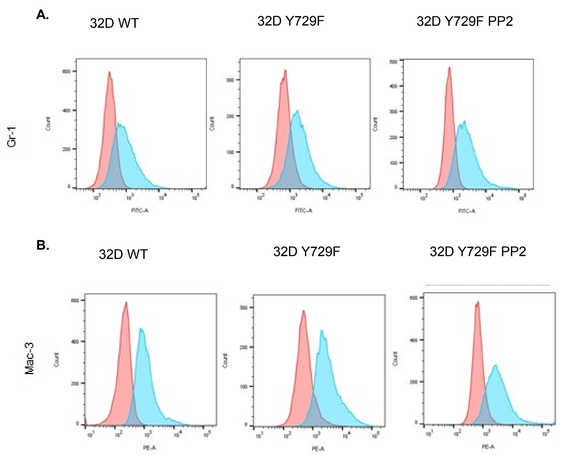
(A-C) Mutant 32D Y729F cells were utilized in this experiment. In the control (Ctr) samples the cells were left untreated. Samples labeled with the hour marker alone were only treated with G-CSF for that prescribed time. However, samples with the "P" to the right of the hour marker were treated with G-CSF as well as PP2 for the time denoted above. Egr-1 and c-Fos activation were both examined by Western Blot analysis using β-actin as a control.

Figure 6: Effects of PP2 on the Expression of Monocyte and Neutrophil Differentiation Markers



(A-E) 32D Y729F cells were cultured for 3 days in G-CSF. The PP2 samples contained 32D Y729F cultured in both G-CSF and PP2 for 3 days. RT-PCR was utilized to quantify the relative mRNA expression level within each sample type. The M-CSF and Mmp-12 genes are monocyte differentiation markers. ELANE, LF, and MPO genes are neutrophil differentiation markers.

Figure 7: Effects of PP2 on Surface Expression of Gr-1 and Mac-3



(A) All samples were stimulated over 3 days with G-CSF with one of the samples being treated with PP2 as denoted above, Gr-1 antibody was used to probe for neutrophils during flow cytometry analysis. **(B)** All samples were stimulated with G-CSF over 3 days with one of the samples being treated with PP2 as shown above, Mac-3 antibody was used to probe for monocytes during flow cytometry analysis.

Conclusions

1. PP2 treatment partially rescued morphological differentiation of neutrophils.
2. PP2 treatment in 32D Y729F cells reduced occurrence of cell attachment.
3. However, the expression of monocyte surface markers was increased upon PP2 treatment..
4. Although activation Erk1/2 was weakened in 32D Y729F cells compared to WT 32D cells, the overall strength of c-Fos and Egr-1 showed little difference following PP2 treatment.
5. Further experiments must be conducted to understand the role of SFK and Mek inhibitors in G-CSF-induced signaling.

Acknowledgements

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