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Supplemental Data

# miR-155 Is Associated with the Leukemogenic Potential of the Class IV Granulocyte Colony Stimulating Factor Receptor in CD34+ Progenitor Cells

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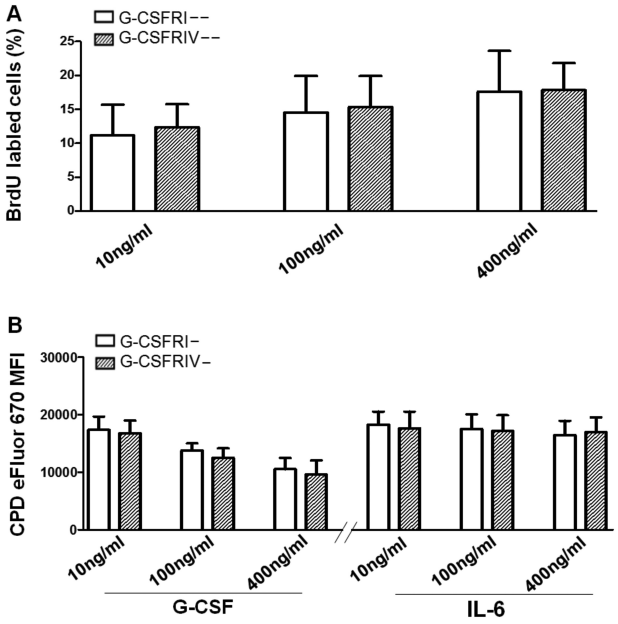
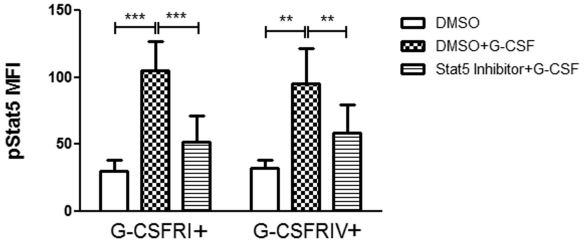
**Supplemental Table S1.** Primers and probes used for real-time PCR.

| Gene | Designation | Sequence (5′→3′) |
|---|---|---|
| *GAPDH* | TaqMan endogenous control | Life Technologies |
| *G-CSFRI* | Forward primer | GACTGTGTCTTTGGGCCACT |
| | Reverse primer | TTAAGAGGCAGGCCCAAGAAG |
| | TaqMan probe | AACCCCAGGAAGCCCTA |
| *G-CSFRIV* | Forward primer | CAGCCCCAATCCCAGTCT |
| | Reverse primer | GGAGCATGATCTGGTCCTTAAAGT |
| | TaqMan probe | TCAGGCTGGGCCTCC |
| *GFI-1* | TaqMan gene exression | Life Technologies |
| *hsa-miR-155-5p* | TaqMan MicroRNA Assay | UUAAUGCUAAUCGUGAUAGGGGU |
| *PU.1* | TaqMan gene exression | Life Technologies |
| *TP53INP1* | TaqMan gene exression | Life Technologies |
| *U6 snRNA* | Control miRNA Assay | Life Technologies |




**Supplemental Figure S1.** G-CSFR non-transduced cells demonstrate the same proliferation potential in presence of G-CSF. CD34+ HSPCs were transduced with a lentiviral vector containing either G-CSFRI or G-CSFRIV encoding sequences. (A) $1x10^5$ unsorted cells were stimulated with 10 ng/mL, 100 ng/mL and 400 ng/mL G-CSF for 1 d and incorporated with BrdU for 3 h followed by anti-BrdU antibody staining and FACS analysis. Anti-CD114 was used to distinguish G-CSFR overexpressing and non-expressing cells. Graph displays percentage of BrdU-labeled cells of total G-CSFRI-- and G-CSFRIV-- cells. (B) $1x10^5$ cells were labeled with (CPD) eFluor 670 and stimulated with indicated concentrations of G-CSF or IL-6 for 3 d followed by FACS analysis. GFP expression was used to distinguish G-CSFR overexpressing and non-transduced cells. Graph displays MFI of CPD eFluor 670 on G-CSFRI- and G-CSFRIV- cells. The data shown are mean ± SD of 6 independent experiments. Statistically significant differences were calculated using a Student two-tailed *t* test. HSPCs: hematopoietic stem and progenitor cells. G-CSFRI--: G-CSFRI non-expressing. G-CSFRIV--: G-CSFRIV non-expressing. MFI: mean fluorescence intensity. CPD: cell proliferation dye. G-CSFRI-: G-CSFRI non-transduced. G-CSFRIV-: G-CSFRIV non-transduced. Statistically significant differences were calculated using a two-tailed Student *t* test.




**Supplemental Figure S2.** The Stat5 inhibitor decreases Stat5 phosphorylation. CD34+ HSPCs were transduced with a lentiviral vector encoding for either G-CSFRI or G-CSFRIV sequences. $1x10^5$ serum-starved cells were stimulated with 100 ng/mL G-CSF with DMSO as control or with a Stat5 inhibitor for 20 min and Stat5 phosphorylation was evaluated using BD Phosflow technology. Graph displays MFI of G-CSF induced phosphorylated Stat5 on G-CSFRI+ and G-CSFRIV+ HSPCs. The data shown are mean ± SD of 5 independent experiments. Statistically significant differences were calculated using a two-tailed Student *t* test and are shown with asterisks (**$p < 0.01$ and ***$p < 0.001$). HSPCs: hematopoietic stem and progenitor cells. G-CSFRI+: G-CSFRI overexpressing. G-CSFRIV+: G-CSFRIV overexpressing. MFI: mean fluorescence intensity.