Pegylated granulocyte colony-stimulating factor mobilizes CD34+ cells with different stem and progenitor subsets and distinct functional properties in comparison with unconjugated granulocyte colony-stimulating factor

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Quantification, normalization and statistical analyis

We utilized the *affy* package of functions of statistical scripting language 'R' integrated into the Bioconductor project (*http://www.bioconductor.org/*) as described previously.[10] Using histograms of perfect match intensities, 5' to 3' RNA degradation side-by-side plots, or scatter plots, we estimated the quality of probes and hybridizations. To normalize raw data, we used a method of variance stabilizing transformations (VSN).[11]

To compare the normalized data from Peg-G-CSF-mobilized CD34+ cells and G-CSF-mobilized CD34+ cells, we used the significance analysis of microarrays (SAM) algorithm v2.23 (*http://www-stat.stanford.edu/_tibs/SAM/*), which contains a sliding scale for a false discovery rate (FDR) of significantly up- and downregulated genes. All data were permuted 1000 cycles by using the two classes, unpaired data mode of the algorithm. As a cut-off for significance an estimated FDR of 5% was chosen by the tuning parameter delta of the software.

The significance level for each gene was given by the q-value (lowest FDR at which the gene is called significant). Moreover, a cut-off for fold-change of differential expression of 1.2 was used. Hierarchical cluster analysis was performed by the hclust function of the *R* software using an average linkage clustering algorithm.

Supplementary Table 1.

Selection of genes with significantly differential expression levels (q-value <5%) in CD34$^+$ cells after mobilization with either Peg-G-CSF or G-CSF

Gene Title	Fold Change Peg-G-CSF/G-CSF	Gene Symbol
Stem cells		
homeo box B2	1.39	HOXB2
homeo box A4	1.20	HOXA4
homeo box A5	1.31	HOXA5
homeo box A9	1.48	HOXA9
homeo box A10	1.28	HOXA10
pre-B-cell leukemia transcription factor 3	1.23	PBX3
myeloid ecotropic viral integration site 1 homolog	1.56	MEIS1
Myeloid/lymphoid or mixed-lineage leukemia	1.35	MLL
aldehyde dehydrogenase 1 family, member A1	1.28	ALDH1A1
prominin 1	1.46	PROM1
GATA binding protein 3	1.37	GATA3
Erythropoiesis		
hemoglobin, alpha 2 /// hemoglobin, alpha 2	0.06	HBA2
hemoglobin, alpha 1 /// hemoglobin, alpha 2	0.07	HBA1 /// HBA2
hemoglobin, beta	0.13	HBB
hemoglobin, delta /// hemoglobin, delta	0.50	HBD
Kruppel-like factor 1 (erythroid)	0.69	KLF1
CD36 antigen (collagen type I receptor, thrombospondin receptor)	0.50	CD36
erythropoietin receptor	0.83	EPOR
PRKC, apoptosis, WT1, regulator	1.41	PAWR
Myelopoiesis		
defensin, alpha 1 /// defensin, alpha 3	0.08	DEFA1 /// DEFA3
defensin, alpha 4, corticostatin	0.51	DEFA4
matrix metalloproteinase 8 (neutrophil collagenase)	0.26	MMP8
matrix metalloproteinase 9 (gelatinase B)	0.28	MMP9
CCAAT/enhancer binding protein (C/EBP), beta	0.38	CEBPB
CCAAT/enhancer binding protein (C/EBP), delta	0.38	CEBPD
Regulation of differentiation		
inhibitor of DNA binding 2, dominant negative helix-loop-helix protein	0.53	ID2
growth arrest and DNA-damage-inducible, gamma	0.82	GADD45G
Cell proliferation		
cyclin D2	1.34	CCND2
hepatic leukemia factor	1.72	HLF
RAB GTPase activating protein 1	1.44	RABGAP1
retinoblastoma binding protein 6	1.20	RBBP6
TGFB1-induced anti-apoptotic factor 1 /// myosin XVIIIA	1.53	TIAF1 /// MYO18A
tumor necrosis factor (ligand) superfamily, member 4 1, 34kDa)	1.37	TNFSF4
membrane-spanning 4-domains, subfamily A, member 4	0.35	MS4A4
membrane-spanning 4-domains, subfamily A, member 3	0.63	MS4A3
cyclin D3	0.69	CCND3
protein kinase C, eta	1.23	PRKCH
protein kinase, cAMP-dependent, catalytic, beta	1.61	PRKACB