To the editor:

HemaExplorer: a Web server for easy and fast visualization of gene expression in normal and malignant hematopoiesis

The HemaExplorer Web server allows for an easy display of mRNA expression profiles for query genes in murine and human hematopoietic stem and progenitor cells that represent consecutive developmental stages along the myeloid differentiation pathway. In addition, it provides the ability to compare gene expression levels in human acute myeloid leukemia (AML) cells with expression levels in normal human hematopoietic stem cells and hematopoietic progenitor cells. The Web server is freely available at http://servers.binf.ku.dk/hemaexplorer/.

The HemaExplorer Web server can take 1 gene as query and provides a plot of the expression of the gene in both hematopoietic stem and progenitor populations as well as in mature lineages. Alternatively, a query of 2 genes depicts their relationship in a scatter plot. Currently the database contains options for the human hematopoietic system, human AML, and the murine hematopoietic system (see Figure 1).

The Web server accepts gene symbol aliases as long as they are unambiguous, for example the query "p53" will be interpreted as *TP53*. The Web server will provide output plots for download in ready-to-publish quality. In addition, the raw data for the query gene is available for download. There is also an advanced option allowing for selection of the cell types presented by the output plot.

The database was build using manually curated public available gene expression datasets from multiple studies all generated on the Affymetrix platform (Human 133UA and 133UB and 133.Plus2 microarrays; Mouse Genome 430 2.0 Array).[1-9]

To correct for batch effect the datasets were sorted by platform and laboratory, before RMA normalization and subsequent processing with ComBat.[10] As several platforms are represented in the dataset, only the overlapping probe sets were retained for further processing. Importantly, fold changes for AML samples were computed using a novel normalization method that first identifies the nearest normal counterpart to the individual AML sample and use this to compute gene expression changes in the AML sample (N.R., J.J., K.T.M., O.W. and B.T.P, unpublished data, May 2012). For genes detected by more than 1 probe sets on the microarrays, gene expression levels are presented by the probe set with the highest mean expression level; data from all available array probe sets are available for download.

In summary, the HemaExplorer Web server will provide researchers with a powerful tool to check expression levels for genes of interest in normal hematopoiesis as well as AML.

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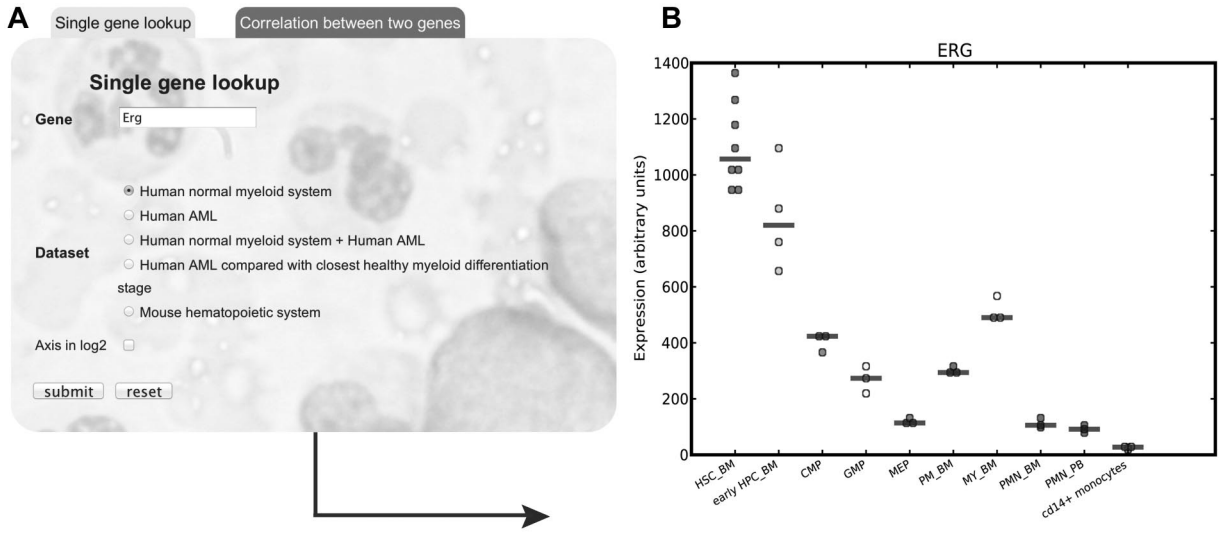

Figure 1. Example of gene query to the HemaExplorer Web server. (A) Web server interface for single gene lookup with gene query, options for dataset and scale of axis. (B) Output plot from the Web server displaying gene expression for the *ERG* transcription factor in the normal myeloid system. CMP indicates common myeloid progenitor cell; GMP, granulocyte monocyte progenitors; HSC_BM, hematopoietic stem cells from bone marrow; MEP, megakaryocyte-erythroid progenitor cell; MY_BM, myelocyte from bone marrow; PMN_BM, polymorphonuclear cells from bone marrow; PMN_PB, polymorphonuclear cells from peripheral blood; PM_BM, promyelocyte from bone marrow; and early HPC_BM, hematopoietic progenitor cells from bone marrow.

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Contribution: N.R., K.T.-M., and B.P. developed the original idea; F.O.B. made the Web server; B.K., N.R, and K.T.M. conducted data collection; F.O.B., N.R., K.T.M., and B.P. wrote the letter; and O.W. and L.J.J. reviewed the Web server.

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To the editor:

Evidence for miRNA expression differences of HIV-1–positive, treatment-naive patients and elite suppressors: a re-analysis

Bignami et al recently reported on miRNA profiles of treatment-naive HIV-1 patients (naive), elite suppressors (éLTNP or ES), and multiply-exposed, uninfected individuals (MEUs).[1] The authors identified only 1 putative miRNA expression difference between naive and ES and did not confirm results of earlier studies of miRNAs in HIV-1 susceptible cells and infected patients.[2-5] After re-analyzing the data (Gene Expression Omnibus series GSE33514), we suggest that technical and experimental design issues, including comparisons of patients with a single control sample, may have inappropriately affected the authors' conclusions. Our alternative examination tended to corroborate previous findings.

We posit that the original conclusions may derive from analysis issues and batch effects. There were significant differences in the number of miRNAs detected in MEU or control versus naive/ES (Figure 1A; $P < .001$). Further, when we used the specified U6 normalization and exclusion criteria, 70 miRNAs of < 150 detected consistently in MEU had significant apparent expression differences ($P < .05$ with multiple comparison correction). Each was rarer in MEUs than infected patients. A uniformly 1-way directionality of expression differences is unusual and inconsistent with previous work[2]; Figure 1B details additional issues. We conclude that technical factors of RNA integrity and processing (no quality control assessments were provided), measurement, and analysis likely affected results.

The titular case for a retroviral exposure signature is tenuous as presented. First, only unprotected sex suggested exposure of MEUs to HIV-1. The authors did not specify whether individuals had HIV-1–positive sex partners or if these partners were taking antiretrovirals. It seems unusual to define a patient class by conjecture. Second, the normalizing RNA, U6 was not "relatively stable" in these samples, but varied 14-fold. The highest threshold cycle (Ct) was in the healthy donor sample (Figure 1C), likely skewing results. Third, 5 members of the proposed octapartite exposure signature[1] (Figure 2A) failed the authors' inclusion criterion (expression in $> 70\%$ of at least 1 patient group); each was undetected in 13-16 of 17 patient samples. The other 3 were considered up-regulated in patients only because they were undetected in the single control PCR well, insufficient to conclude absence or calculate accurate fold changes. Many such "undetected" miRNAs were found in previous studies of healthy $CD4^+$ T cells.[6,7] Finally, no meaningful conclusions can be drawn from comparisons made with a single control sample, pooled or not. The authors' gp120 experiments,[1] (Figure 3) also lack biologic replicates. With an $n = 1$, assertions of a retroviral exposure signature and a gp120-mediated miRNA profile shift are preliminary.

Contrasting with the authors' conclusions, alternative analyses using quantile normalized data from miRNAs expressed in the majority of samples revealed multiple naive-to-ES differences. Although confidence in these results remains attenuated by the observations above and would be bolstered by verification with technical replicates and/or different measurement platforms, it is