Data in Brief

Transcriptome comparison of distinct osteolineage subsets in the hematopoietic stem cell niche using a triple fluorescent transgenic mouse model

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ABSTRACT

The bone marrow niche is recognized as a central player in maintaining and regulating the behavior of hematopoietic stem and progenitor cells. Specific gain-of and loss-of function experiments perturbing a range of osteolineage cells or their secreted proteins had been shown to affect stem cell maintenance (Calvi et al, 2003 [1]; Stier et al., 2005 [2]; Zhang et al., 2003 [3]; Nilsson et al., 2005 [4]; Greenbaum et al., 2013 [5]) and engraftment (Adam et al., 2006, 2009 [6,7]). We used specific in vivo cell deletion approaches to dissect the niche cell-parenchymal cell dependency in a complex bone marrow microenvironment. Endogenous deletion of osteocalcin-expressing (Ocn^+) cells led to a loss of T immune cells (Yu et al., 2015 [8]. Ocn^+ cells express the Notch ligand DLL4 to communicate with T-competent progenitors, and thereby ensuring T precursor production and expression of chemotactic molecules on their cell surface for subsequent thymic seeding. In contrast, depletion of osterix-expressing (Osx^+) osteoprogenitors led to reduced B immune cells. These distinct hematopoietic phenotypes suggest specific pairing of mesenchymal niche cells and parenchymal hematopoietic cells in the bone marrow to create unique functional units to support hematopoiesis. Here, we present the global gene expression profiles of these osteolineage subtypes utilizing a triple fluorescent transgenic mouse model ($OsxCre^+;Rosa-mCh^+;Ocn:Topaz^+$) that labels Osx^+ cells red, Ocn^+ cells green, and Osx^+Ocn^+ cells yellow. This system allows isolation of distinct osteolineage subsets within the same animal by flow cytometry. Array data that have been described in our study [8] are also publically available from NCBI Gene Expression Omnibus (GEO) with the accession number GSE66042. Differences in gene expression may correlate with functional difference in supporting hematopoiesis.

Specifications

Organism/cell line/tissue	*Mus musculus*
Sex	Females
Sequencer or array type	Affymetrix Mouse Genome 430A 2.0 Array
Data format	Raw data normalized
Experimental factors	Transcriptome comparison of Osx^+ and Ocn^+ osteolineage cells.
Experimental features	In the $OsxCre^+;Rosa-mCh^+;Ocn:Topaz^+$ mouse model, Osx^+ cells were labeled red, Ocn^+ cells were green, and Osx^+Ocn^+ cells were yellow within the same animal. This system allows isolation of osteolineage subsets within the same animal by flow cytometry and subject to microarray comparison of global gene expression. Mice were 8–12 weeks old. Three replicates were used for each experimental condition.
Consent	Allowed to reuse citing original author.
Sample source location	NA

1. Direct link to deposited data

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE66042.

2. Experimental design, materials and methods

2.1. Mouse models

Eight to twelve-week-old mice were used for all the experiments unless otherwise specified. Mice carrying a fusion of Cre recombinase with a modified estrogen receptor (ERt) under the control of the murine *Osterix* promoter (Osx-CreERt2 [9]), hereafter called OsxCre, were crossed with mice bearing a Rosa26-loxP-stop-loxP-mcherry (Rosa-mCh) transgene (OsxCre;Rosa-mCh). Administration of the estrogen analog, 4-hydroxy-tamoxifen (4-OHT) to $OsxCre^+;Rosa-mCh^+$ mice resulted in selective Cre activation in Osx-expressing cells followed by

excision of the stop cassette and subsequent production of the mCherry fluorophore. The red fluorescent protein marks cells expressing Osx at the time of 4-OHT injection and their progeny. These mice were crossed with mice expressing the green fluorescent protein, Topaz, driven by the osteocalcin promoter (Ocn:Topaz). In this triple transgenic model (OsxCre$^+$;Rosa-mCh$^+$;Ocn:Topaz$^+$), the osteocalcin-expressing cells are green, the osterix expressing cells (and their descendants) are red and cells expressing both produce yellow. All animal usage and procedures performed were approved by the Institutional Animal Care and Use Committee of Massachusetts General Hospital.

2.2. Flow cytometry

For each mouse, tibiae, femurs, iliac crests, spines, ulnae, radii, and humeri were collected for bone marrow cells. Muscles and soft tissue were thoroughly removed from the bones that were flushed to remove the bone marrow content. Bone fragments were cut into small fragments and transferred into a pre-warmed collagenase solution (Stemcell Technologies), for 1 h at 37° with vigorous shaking. The solution was then filtered through a 40 μm filter and the flow-through was pelleted and further processed for FACS sorting or RNA extraction.

2.3. RNA isolation

Cells were sorted in 750 μl of TRIzol® (Invitrogen) and RNA was extracted according to the instructions of the manufacturer.

2.4. Microarrays

Osx$^+$, ++ and Ocn$^+$ cells were sorted at 6 days post tamoxifen injection from 4 to 6 weeks adults by using the FACSAria. RNA was extracted using the TRIzol® (Invitrogen) according to the manufacturer's instructions. Samples were further processed by the NuGen Ovation V2 laboratory Process in the microarray core facility of Dana-Farber Cancer Institute. Briefly, first stand cDNA was prepared from total RNA using a DNA/RNA chimeric primer and a reverse transcriptase. The resulting double stranded cDNA with a unique heteroduplex at the 5' end of the antisense strand was amplified using SPIATM amplification, a repeated process of SPIATM DNA/RNA primer hybridization, DNA replication, strand displacement and RNA cleavage which resulted in a rapid accumulation of cDNA with sequence complementary to the original RNA. The SPIATM amplified cDNA was purified using the Zymo Research DNA Clean & ConcentratorTM system. The purified cDNA was fragmented through a chemical and enzymatic process and labeled via enzymatic attachment of a biotin-labeled nucleotide to the 3′-hydroxyl end of the fragmented cDNA. The biotinylated cDNA was added to a hybridization solution containing several biotinylated control oligonucleotides (for quality control), and hybridized to the Mouse430A microarray chip overnight at 45 °C. The chips were then transferred to a fluidics instrument that performs washes to remove cDNA that has not hybridized to its complementary oligonucleotide probe. The bound cDNA was then fluorescently labeled using phycoerythrin-conjugated streptavidin (SAPE); additional fluors were then added using biotinylated anti-streptavidin antibody and additional SAPE. Each cDNA bound at its complementary oligonucleotide was excited using a confocal laser scanner, and the positions and intensities of the fluorescent emissions were captured. These measures provide the basis of subsequent biostatistical analysis. Standard QA/QC analyses involved chip analysis with the assayQualityMetrics BioConductor package and found no significant quality issues with any of the chips, as determined by (among other methods) visual inspection, intensity distributions or RNA degradation plots.

2.5. Quality assessment

Standard QA/QC analyses involved chip analysis with the assayQualityMetrics BioConductor package and found no significant quality issues with any of the chips, as determined by (among other methods) visual inspection, intensity distributions or RNA degradation plots. The data was background corrected and normalized with RMA (Robust Multichip Average) using the "affy" BioConductor package. Values in the data matrix represent log2 normalized intensity values.

3. Differential expression quantification and classification

Supervised learning or class prediction methods were used for molecular classification and pattern recognition. This analysis involves selecting the features (genes) most correlated with a phenotypic distinction of interest. These features or "marker genes" are biologically interesting in themselves but they can also be used as the input of a classification algorithm that uses existing "labeled" samples to build a model to predict the labels for future samples. Genes correlated with a binary class distinction, for example a morphological or clinical phenotype, is directly identified and selected by using a "distance" metric, for example t-test statistic = (mA − mB) ÷ (s2A + s2B) [m and s are the means and std. dev. per class] or Signal to noise ratio = (mA − mB) / (sA + sB) [m and s are the means and std. dev. per class].

References

[1] L.M. Calvi, et al., Osteoblastic cells regulate the haematopoietic stem cell niche. Nature 425 (2003) 841–846.
[2] S. Stier, et al., Osteopontin is a hematopoietic stem cell niche component that negatively regulates stem cell pool size. J. Exp. Med. 201 (2005) 1781–1791.
[3] J. Zhang, et al., Identification of the haematopoietic stem cell niche and control of the niche size. Nature 425 (2003) 836–841.
[4] S.K. Nilsson, et al., Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. Blood 106 (2005) 1232–1239.
[5] A. Greenbaum, et al., CXCL12 in early mesenchymal progenitors is required for haematopoietic stem-cell maintenance. Nature 495 (2013) 227–230.
[6] G.B. Adams, et al., Haematopoietic stem cells depend on Galpha(s)-mediated signalling to engraft bone marrow. Nature 459 (2009) 103–107.
[7] G.B. Adams, et al., Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. Nature 439 (2006) 599–603.
[8] V.W. Yu, et al., Specific bone cells produce DLL4 to generate thymus-seeding progenitors from bone marrow. J. Exp. Med. 212 (2015) 759–774.
[9] C. Maes, et al., Osteoblast precursors, but not mature osteoblasts, move into developing and fractured bones along with invading blood vessels. Dev. Cell 19 (2010) 329–344.