Quantitative analysis by next generation sequencing of hematopoietic stem and progenitor cells (LSK) and of splenic B cells transcriptomes from wild-type and Usp3-knockout mice

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A B S T R A C T

The data described here provide genome-wide expression profiles of murine primitive hematopoietic stem and progenitor cells (LSK) and of B cell populations, obtained by high throughput sequencing. Cells are derived from wild-type mice and from mice deficient for the ubiquitin-specific protease 3 (USP3; Usp3Δ/Δ). Modification of histone proteins by ubiquitin plays a crucial role in the cellular response to DNA damage (DDR) (Jackson and Durocher, 2013) [1]. USP3 is a histone H2A deubiquitinating enzyme (DUB) that regulates ubiquitin-dependent DDR in response to DNA double-strand breaks (Nicassio et al., 2007; Doil et al., 2008) [2,3]. Deletion of USP3 in mice increases the incidence of spontaneous tumors and affects hematopoiesis [4]. In particular, Usp3-knockout mice show progressive loss of B and T cells and decreased functional potential of hematopoietic stem cells (HSCs) during aging. USP3-deficient cells, including HSCs, display enhanced histone ubiquitination, accumulate spontaneous DNA damage and are hypersensitive to...
To address whether USP3 loss leads to deregulation of specific molecular pathways relevant to HSC homeostasis and/or B cell development, we have employed the RNA-sequencing technology and investigated transcriptional differences between wild-type and Usp3Δ/Δ LSK, naïve B cells or in vitro activated B cells. The data relate to the research article “Tight regulation of ubiquitin-mediated DNA damage response by USP3 preserves the functional integrity of hematopoietic stem cells” (Lancini et al., 2014) [4]. The RNA-sequencing and analysis data sets have been deposited in NCBI’s Gene Expression Omnibus (Edgar et al., 2002) [5] and are accessible through GEO Series accession number GSE58495 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE58495). With this article, we present validation of the RNA-seq data set through quantitative real-time PCR and comparative analysis.

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Data can be used in the development of further experiments aimed at addressing how the ubiquitin-dependent DNA damage response pathway impact on hematopoietic stem cell biology.

1. Data

mRNA profiles of murine populations of lineage negative cKit+ SCA1+ (LSK) hematopoietic progenitors, and naïve or activated B cell populations from 8 weeks-old wild-type (WT) and Usp3-deleted (Usp3Δ/Δ) mice were generated by deep sequencing using Illumina Hiseq2000. Here we present validation of the datasets by comparative analysis and qRT-PCR (Fig. 1). qRT-PCR validation of the RNA-seq on LSK cells was performed using SYBR Green assays.

2. Experimental design and materials and methods

2.1. Experimental design

The cells used as a source for RNA-seq were lineage negative, cKit+, Sca1+ (LSK) hematopoietic stem and progenitor cell population purified by fluorescence-activated cell sorting (FACS) from freshly isolated bone marrow, FACS-sorted naïve B cells from spleens (CD19+) and activated B cells harvested and FACS sorted after 4 days stimulation with lipopolysaccharide (LPS) in culture. Two biological replicas were analyzed. For each experiment WT n=4, Usp3Δ/Δ n=4 mice were used. FACS-sorted cells from individual animals were pooled and subjected to deep sequencing. We assigned about 8–16 million reads per sample uniquely to a gene of the mouse reference genome (mm9). We identified 23,429 genes in the LSKs, naive B cells and activate B cells of WT and Usp3Δ/Δ mice using TopHat in combination with HTSeq-count. The raw data files that were used in the validation/analysis presented here and in the analysis and interpretation in [4] have been deposited in the NCBI’s Gene Expression Omnibus [5] database with the GEO Series accession no. GSE58495 (Fig. 1).

2.2. Lin-Sca1+ cKit+ (LSK) hematopoietic progenitor isolation

Bone marrow cells were extracted from 8 weeks-old wild-type (WT) and Usp3 knockout (Usp3Δ/Δ) mice (MGI:5490048; B6.129P2(FVB)-Usp3o tm1.1Nki4;[4]). For cell sorting of bone marrow (BM) LSK sub-populations [9,10], depletion of lineage+ cells was performed using Biotin MicroBeads (130-090-485, Macs, Miltenyi biotec) and magnetic columns (130-042-401, Macs, Miltenyi biotec). Cell were then directly stained with fluorochrome-conjugated antibodies against Sca1 (Pacific blue) (Biolegend), c-Kit (APC)(BD) and Strep (APC/CY7) (Souther Biotech). Cell sorting was performed with FACSaria (BD Biosciences). All animal experiments comply with local and international regulations and ethical guidelines and have been authorized by our local experimental animal committee at The Netherlands Cancer Institute (DEC-NKI).

2.3. B cell isolation

B cells were extracted from 8 weeks-old WT and Usp3Δ/Δ mice. Naive splenic B cells were obtained by CD43 depletion using biotinylated anti CD43 (Clone S7, BD Biosciences), and the IMag system (BD Biosciences), as described by the manufacturer. Naïve B cells were directly FACS sorted with fluorochrome-conjugated antibody specific for CD19 (APC), or cultured in vitro for four days in IMDM+8%FBS and 50 μg/ml Escherichia coli LPS (055:B5, Sigma) to obtain activated B cells, followed by sorting. Two independent experiments were performed. Cell sorting was performed by FACSaria (BD Biosciences).
2.4. RNA-seq gene expression analysis

For gene expression analysis, LSKs (FACS sorted from freshly isolated BM), naïve splenic B cells (fresher FACs sorted) or FACs sorted LSP-activated B cells were used. N=4 Usp3Δ/Δ and N=4 WT littermates (8 weeks-old). Cells from individual animals were pooled and total RNA was extracted. Samples were prepared using TruSeq protocols and standard Illumina sample preparation protocols and RNA-seq was performed on an Illumina Hiseq2000 machine at the NKI Genomics Core Facility.
The sequence reads that passed quality filters were mapped to mm9 with TopHat version 2.0.3 and the gene expressions were calculated using HTSeq-count. The expression levels are normalized to 10 million reads per sample (GSE58495_diffexp_LSK.txt.gz file). Differential expression was performed using the R package DEGseq (GSE58495_norm_gene_exp_10mil.txt.gz). Upon differential expression analysis all values were added with 1. Genes that had no expression in both samples were removed.

2.5. Quantitative real-time (qRT-)PCR

Total RNA was extracted using Trizol reagent (Life technologies) and cDNA was prepared using Superscript II RT and oligod(T)n primers (Life technologies). qRT-PCR was performed on a StepOne-Plus Real-Time PCR system (Applied Biosystems) using SYBR Green PCR mastermix (Applied Biosystems). The amount of target, normalized to an endogenous reference (TBP or beta actin) was calculated by: \(2^{-\Delta\Delta CT}\).

Primer sequences used in validation of RNA seq analysis (Supplementary Table S1) are available upon request.

2.6. Statistics

Statistical analysis was performed by Student t test or Pearson correlation analysis in Prism 6.

Conflict of interest

The authors declare no competing financial interests.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2015.12.049.

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