Transcriptional profiling of Foxo3a and Fancd2 regulated genes in mouse hematopoietic stem cells

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Abstract

Functional maintenance of hematopoietic stem cells (HSCs) is constantly challenged by stresses like DNA damage and oxidative stress. Foxo factors particularly Foxo3a function to regulate the self-renewal of HSCs and contribute to the maintenance of the HSC pool during aging by providing resistance to oxidative stress. Fancd2-deficient mice had multiple hematopoietic defects including HSC loss in early development and in response to cellular stresses including oxidative stress. The cellular mechanisms underlying HSC loss in Fancd2-deficient mice include abnormal cell cycle status loss of quiescence and compromised hematopoietic repopulating capacity of HSCs. To address on a genome wide level the genes and pathways that are impacted by deletion of the Fancd2 and Foxo3a, we performed microarray analysis on phenotypic HSCs (Lin− cKit+Sca-1+CD150+CD48−) from Fancd2 single knockout Foxo3a single knockout and Fancd2−/− Foxo3a−/− double-knockout (dKO) mice. Here we provide detailed methods and analysis on these microarray data which has been deposited in Gene Expression Omnibus (GEO): GSE64215.

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Experimental design, materials and methods

Hematopoietic stem cells isolation

Primary mouse hematopoietic stem cells were derived from 2 months old mice as previously described [1]. Briefly, the bone marrow fraction was dissociated from the femurs and tibias by flushing. The HSC subpopulation was stained with antibodies for Lin−, c-Kit, Sca-1, CD150 and CD48. Flow cytometry were performed on an LSRII (BD) to isolate the HSCs population (Lin− cKit+Sca-1− CD150− CD48−).

RNA extraction

Total RNA was extracted from HSCs of WT, Fancd2−/−, Foxo3a−/− and Fancd2−/− Foxo3a−/− mice by using the RNeasy Kit (Qiagen) following the manufacturer’s procedure. RNA quality and quantity were determined by using a NanoDrop 1000 (Thermo Scientific) and Agilent 2100 bioanalyzer (RNA Nano Chips, Agilent). The RNA integrity number (RIN) of the RNA samples ranged from 7 to 10 for the microarray analysis. For each sample approximately 1 nanogram of total RNA was used.

Sample labeling and scanning

The Ovation Pico WTA System v2 (NuGEN) was used to prepare target cDNA. The Encore Biotin Module (NuGEN) chemically fragmented
and labeled the target cDNA. 3.5 μg of fragmented cDNA was hybridized to a Mouse Gene 2.0 ST arrays (Affymetrix). Probe arrays were incubated at 45 °C for 18 h in the hybridization Oven 640 (Affymetrix) rotating at 60 rpm. Probe arrays were washed and stained using the Fluidics Station 450 (Affymetrix), utilizing the fluids protocol FS450-0002. The stain and antibody solutions are produced by Affymetrix and contained in the Genechip Hybridization Wash and Stain Kit. GeneChips were scanned using the Affymetrix GeneChip Scanner 3000 7G.

Data normalization and filtering

The .cel files were created by Command Console (Affymetrix), and then were analyzed by Expression Console (Affymetrix) to create the .chp files. The Gene Expression Core uses this program to create normalized RMA Data files for each sample. These analysis files permit the core personnel to examine a number of quality control factors, to ensure the chip hybridizations were OK (Fig. 1).

Discussion

Here we describe the genome-wide transcriptional profiling of Fancd2 and Foxo3a regulated genes in mouse hematopoietic stem cells. This data set is the first microarray analysis to identify target genes that are differentially expressed and regulated by Fancd2 and Foxo3a in mouse HSCs. Foxo3a transcription factor is known to play a role in anti-oxidant stress and in HSC self-renewal [2,3]. Fancd2 is critical in DNA damage repair of double-stranded breaks [4,5]. Accordingly, our results reveal that Foxo3a and Fancd2 target genes are enriched in pathways involved in DNA repair, DNA binding and cell cycle checkpoints. These findings reveal functional interaction between the FA DNA repair pathway and the FOXO3a stress response pathway in HSC maintenance and may suggest new targets for therapeutically exploring the pathogenic role of stress-induced HSC exhaustion in blood diseases.

References

[1] X. Li, J. Li, A. Wilson, J. Sipple, Q. Pang, Fancd2 is required for nuclear retention of Foxo3a in hematopoietic stem cell maintenance. J. Biol. Chem. 290 (2015) 2715–2727.
[2] K. Miyamoto, K.Y. Araki, K. Naka, et al., Foxo3a is essential for maintenance of the hematopoietic stem cell pool. Cell Stem Cell 1 (2007) 101–112.
[3] D. Marinkovic, X. Zhang, S. Yalcin, J.P. Luciano, C. Brugnara, et al., Foxo3 is required for the regulation of oxidative stress in erythropoiesis. J. Clin. Invest. 117 (2007) 2133–2144.
[4] J. Li, W. Du, S. Maynard, P.R. Andreasen, Q. Pang, Oxidative stress—specific interaction between FANCD2 and FOXO3A. Blood 115 (2010) 1545–1548.
[5] K. Parmar, J. Kim, S.M. Sykes, et al., Hematopoietic stem cell defects in mice with deficiency of Fancd2 or Usp1. Stem Cells 28 (2010) 1186–1195.