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Transcriptome-wide Profiling and Posttranscriptional Analysis of Hematopoietic Stem/Progenitor Cell Differentiation toward Myeloid Commitment

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SUMMARY

Hematopoietic stem cells possess lifelong self-renewal activity and generate multipotent progenitors that differentiate into lineage-committed and subsequently mature cells. We present a comparative transcriptome analysis of ex vivo isolated mouse multipotent hematopoietic stem/progenitor cells (Lin^{neg}SCA-1^{+}c-KIT^{+}) and myeloid committed precursors (Lin^{neg}SCA-1^{neg}c-KIT^{+}). Our data display dynamic transcriptional networks and identify a stem/progenitor gene expression pattern that is characterized by cell adhesion and immune response components including kallikrein-related proteases. We identify 498 expressed lncRNAs, which are potential regulators of multipotency or lineage commitment. By integrating these transcriptome with our recently reported proteome data, we found evidence for posttranscriptional regulation of processes including metabolism and response to oxidative stress. Finally, our study identifies a high number of genes with transcript isoform regulation upon lineage commitment. This in-depth molecular analysis outlines the enormous complexity of expressed coding and noncoding RNAs and posttranscriptional regulation during the early differentiation steps of hematopoietic stem cells toward the myeloid lineage.

INTRODUCTION

In the adult hematopoietic system, short-lived mature cells are constantly lost and need to be replaced in order to maintain blood homeostasis (Murphy et al., 2005; Weissman and Shizuru, 2008). This essential task is fulfilled by hematopoietic stem cells (HSCs), which reside in the trabecular areas of the bone marrow (Purton and Scadden, 2007; Till and McCulloch, 1961; Wilson et al., 2009). HSCs possess the highest self-renewal capacity and produce multipotent progenitors (MPPs) with steadily decreasing self-renewal activity (Trumpp et al., 2010; Weissman and Shizuru, 2008). HSCs and MPPs (HSPC) are contained within a compartment immunophenotypically defined as negative for mature blood cell markers (Lin^{-}) and positive for stem cell markers SCA-1 and c-KIT (LS^{+}K; Weissman and Shizuru, 2008). HSPCs eventually commit to more mature lymphoid or myeloid progenitors with increasingly restricted self-renewal and differentiation potential (Graf and Enver, 2009). The myeloid committed progenitor subset (Lin^{-}, SCA-1^{-} and c-KIT^{+}; [LS^{-}K]) comprises common myeloid progenitors (CMPs) as well as more specialized granulocyte-macrophage progenitors (GMPs) and megakaryocyte-erythroid progenitors (MEPs) (Akashi et al., 2000; Pronk et al., 2007), which differentiate toward mature effector cells.

Two crucial aspects of early hematopoiesis are multipotency and lineage commitment (Graf and Enver, 2009; Trumpp et al., 2010). Expression profiling of HSPCs by cDNA microarrays has elucidated important aspects of hematopoietic stem cell biology, including the relevance of the KIT^{-} and Wnt^{-} signaling pathways (Gazit et al., 2013; Kent et al., 2008; Luis et al., 2012; Seita and Weissman, 2010) for multipotency. Transcriptional control networks active in early hematopoiesis have been studied using single-gene expression analysis (Moignard et al., 2013), but their impact on protein levels and posttranscriptional gene expression regulation in HSPCs has not been described.

Recently, transcriptome profiling by next-generation sequencing (NGS; e.g., RNA sequencing [RNA-seq]) has significantly extended the possibilities to study gene expression (Ozsolak and Milos, 2011), which was also used to investigate young versus aged HSCs (Sun et al., 2014). It permits not only the analysis of differential mRNA expression of low abundant regulatory factors, but also the detection of alternative splicing events that can generate different protein isoforms and the identification of noncoding RNAs. Long noncoding RNAs (lncRNAs) (Mercer et al., 2009) are involved in the regulation of gene expression at various levels (Pauli et al., 2011;

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Yoon et al., 2013) and can function as oncogenes or tumor-suppressor genes (Gutschner and Diederichs, 2012). Although efforts have been made to identify and elucidate the roles of lncRNAs in stem cells (Qureshi and Mehler, 2012; Uchida et al., 2012), little is known about the expression of lncRNAs or their functions in hematopoietic stem/progenitors (Paralkar and Weiss, 2013). Further, the advent of improved proteome techniques has enabled in-depth comparative analysis of RNA and protein signatures in diverse systems (Cox and Mann, 2007; Schwanhäusser et al., 2011; Vogel and Marcotte, 2012). Although attempts were made to correlate transcriptome and proteome signatures of hematopoietic immature cells (Spooncer et al., 2008), a comprehensive comparison is still lacking.

We performed a genome-wide RNA-seq analysis of primary multipotent and self-renewing hematopoietic stem/progenitors and myeloid committed precursors. We report robust and reproducible transcriptome data with more than 19,000 quantified genes including more than 1,300 noncoding RNA species. To address how gene expression is regulated in multipotency and commitment, we integrated our RNA-seq data with the recently reported proteome data set of the identical cell populations (Klimmeck et al., 2012). These data sets outline the dynamic expression changes that occur during the transition of stem/progenitors toward myeloid commitment.

RESULTS

Quantitative Transcriptomic Analysis of Hematopoietic Stem and Progenitor Cells

Whole-transcriptome analysis was performed to investigate differences in the gene expression profiles between multipotent hematopoietic stem progenitor cells (HSPCs; LS^+K) and myeloid committed cells (LS^-K) of the mouse bone marrow (Figure 1A). We fluorescence-activated cell sorting (FACS)-sorted 50,000 primary cells of each population in three independent biological experiments (Figure 1B and Figure S1 available online) and enriched for polyadenylated RNA. We generated paired-end libraries and sequenced more than 2×10^8 reads per sample (Figures S1 and S2). Quality-control metrics indicated that the data were reproducible and of high quality (Figures 1C, S1, and S2). We identified the expression of 19,824 genes (Table S1). We classified the quantified genes by RNA categories. As expected after poly(A)-RNA enrichment, the majority of transcripts were categorized as protein-coding genes (78%; 15,474; Figure 1D). In addition, we classified hits to 23 other noncoding RNA categories including pseudogenes (1,783) and lncRNAs (498).

Differential Gene Expression Analysis Reveals Significant Transcriptional Divergence between HSPCs and Myeloid Committed Precursors

We found 3,236 genes to be differentially expressed (false discovery rate [FDR] = 0.05) between multipotent and myeloid committed cells, which indicated a high divergence in transcript levels (Figure 1E; Table S1). Of these, 1,970 genes were highly expressed in HSPCs and 1,266 were higher in LS^-K cells. As expected, transcripts encoding SCA-1, which was used to sort this population, as well as FLT3 and THY1 surface markers (Adolfsson et al., 2005; Weissman and Shizuru, 2008) were preferentially expressed in LS^+K cells (Table S1). In contrast, neutrophil serine proteases *Ctsg* and *Elane* were highly expressed in LS^-K cells (Korkmaz et al., 2008). We validated 24 differentially expressed genes by quantitative real-time PCR, which confirmed the robustness of the data for a wide range of gene expression transcript abundances (from 2 to 2,314,409 of the mean of sequenced fragments; Figure S2D).

To investigate the biological roles of our set of differentially expressed genes, we applied a gene ontology (GO) enrichment analysis, using a stratified approach that controls for expression strength biases (Figure 1F; Table S2). The GO terms overrepresented in LS^+K cells fell into three main categories: immune response (* in Figure 1F; e.g., antigen processing and presentation, inflammatory response), cell adhesion (**; chemotaxis), and transcriptional control (***; negative regulation of transcription). In contrast, metabolism (****; cell redox homeostasis, glycolysis) was significantly overrepresented in myeloid committed cells. Cell cycle (*****) showed a dual pattern with e.g., negative regulation of cell cycle enriched in LS^+K and spindle assembly involved in mitosis enriched in myeloid cells, reflecting the higher proliferative state in the committed progenitors. We determined genes that were uniquely detected in HSPCs but not in LS^-K and vice versa (Figure S3). Although four genes were exclusively detected in LS^-K myeloid progenitors (*Ivl*, *Gm14705*, *Gp6*, *Zfp819*), 69 genes of diverse RNA categories were only detected in LS^+K cells and categorized primarily to cell adhesion and immune system process (e.g., Kallikrein-related peptidases *Klk1*, *Klk9*, and *Klk10*; Figure S3), suggesting particular importance of these processes for the HSPC state. In conclusion, the differential expression pattern for key cellular processes demonstrates a distinct transcriptomic composition of multipotent and myeloid committed cells.

Cell-Cycle Activity Is Tightly Regulated in HSPCs and upon Myeloid Commitment

Next, we analyzed the protein-protein interaction network of the differentially expressed genes related to cell cycle (Figure 2A; ***** in Figure 1F). We found negative regulators of mitosis to be highly expressed in LS^+K cells (*Cdkn1b/p27*),

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