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Transcriptional divergence and conservation of human and mouse erythropoiesis

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Contributed by Harvey F. Lodish, February 3, 2014 (sent for review November 3, 2013)

Mouse models have been used extensively for decades and have been instrumental in improving our understanding of mammalian erythropoiesis. Nonetheless, there are several examples of variation between human and mouse erythropoiesis. We performed a comparative global gene expression study using data from morphologically identical stage-matched sorted populations of human and mouse erythroid precursors from early to late erythroblasts. Induction and repression of major transcriptional regulators of erythropoiesis, as well as major erythroid-important proteins, are largely conserved between the species. In contrast, at a global level we identified a significant extent of divergence between the species, both at comparable stages and in the transitions between stages, especially for the 500 most highly expressed genes during development. This suggests that the response of multiple developmentally regulated genes to key erythroid transcriptional regulators represents an important modification that has occurred in the course of erythroid evolution. In developing a systematic framework to understand and study conservation and divergence between human and mouse erythropoiesis, we show how mouse models can fail to mimic specific human diseases and provide predictions for translating findings from mouse models to potential therapies for human disease.

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to model organisms, in particular mouse models, have been extremely valuable for our understanding of hematopoiesis. The study of this process has provided a paradigm for understanding the molecular mechanisms for lineage-specific cellular differentiation in normal and pathologic states (1–3). Hematopoiesis has long been assumed to be largely conserved based on gross comparative studies among mammals, although the extent of this conservation is unclear. Among the various lineages in the hematopoietic system, erythropoiesis has been extensively studied, given the relevance to human disease and the morphologically distinct stages of erythroid terminal maturation. Forward and reverse genetic approaches in mouse and other model systems have helped define many key regulators of erythropoiesis (4). However, there are examples where aspects of mouse erythropoiesis are inconsistent with human erythropoiesis. For example, humans express a fetal hemoglobin not found in the mouse, and their globin gene regulation pattern differs significantly (5–7). Mutations that impair erythropoiesis in humans are often not faithfully recapitulated in mouse models (8–11). Although specific examples of such differences have been documented, no studies have examined the extent to which mouse erythropoiesis recapitulates the global molecular features of human erythropoiesis.

Hematopoietic stem cells give rise to multiple lineage-committed progenitors and precursors, including the erythroid lineage. The earliest committed erythroid progenitor is the burst-forming unit erythroid (BFU-E) (12, 13). BFU-Es undergo limited self-renewal divisions and differentiate to the colony-forming unit erythroid (CFU-E) (14). Morphologically distinguishable stages of maturation compose the process of terminal erythropoiesis that begins at the CFU-E stage and involves three to six terminal divisions (depending on the species and developmental stage); induction of erythroid-important genes, chromatin condensation; and, in mammals, enucleation. In mammals, the stages of terminal erythropoiesis consist of early (proerythroblasts), intermediate (basophilic erythroblasts), and late (polychromatophilic and orthochromatophilic erythroblasts) erythroid precursors that are morphologically distinct (Fig. S1). Flow cytometric techniques can sort these stages based on surface protein expression, as well as RNA and DNA content (15, 16). Based on surface protein expression, both human and mouse proerythroblasts express modest levels of KIT, high levels of transferrin receptor (CD71), and low levels of glycophorin A (GlyA). Transitioning into the basophilic erythroblast stage, both human and mouse erythroid cells gradually become smaller and begin to express higher levels of GlyA. At the polychromatophilic and orthochromatophilic erythroblast stages, human and mouse erythrocyte cells have reduced expression of CD71, but retain a high level of GlyA. Polychromatophilic and orthochromatophilic erythroblasts contain less RNA and DNA, compared with the earlier stages, and are also smaller (17).

Recent global comparative transcriptional studies in other hematologic lineages, i.e., immune cells, have shown both conservation and divergence (18, 19). As such, it can be argued that the vast extent of divergence may be due to underlying differences in immune cell function and composition (20, 21). In contrast, human and mouse erythroid cells undergo nearly identical maturation processes that are morphologically and functionally similar (17). These studies have suggested specific differences between human and mouse red cell production (erythropoiesis), a global study of such similarities and differences has been lacking. By computationally comparing global gene expression data from adult human and mouse erythroblast precursors representing the distinct stages of maturation, we showed that, while the overall transcriptional landscape has changed, critical erythroid gene signatures and transcriptional regulators have remained conserved. Importantly, these analyses can serve as a tool to integrate data between human and mouse erythropoiesis research, explain why certain human blood diseases are not faithfully recapitulated in mouse models, and highlight hurdles in translating therapeutic findings from mice to humans.

Significance

Mouse models have been instrumental in advancing our understanding of blood cell production. Although many studies have suggested specific differences between human and mouse red cell production (erythropoiesis), a global study of such similarities and differences has been lacking. By computationally comparing global gene expression data from adult human and mouse erythroblast precursors representing the distinct stages of maturation, we showed that, while the overall transcriptional landscape has changed, critical erythroid gene signatures and transcriptional regulators have remained conserved. Importantly, these analyses can serve as a tool to integrate data between human and mouse erythropoiesis research, explain why certain human blood diseases are not faithfully recapitulated in mouse models, and highlight hurdles in translating therapeutic findings from mice to humans.

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structurally indistinguishable (15, 16). However, the extent to which this similarity is matched at the molecular level remains to be determined.

Here we report on a systematic comparative study of human and mouse erythroid precursor gene expression during terminal erythropoiesis using multiple publicly available datasets to quantify and delineate the extent of conservation. Comparing gene expression at matched early, intermediate, and late stages of terminal erythropoiesis between the species as well as the conservation and divergence of transcriptional changes occurring between the developmental stages, we find that, although erythropoiesis seems to be morphologically conserved in the ~65 million years of evolution separating humans and mice, its transcriptional landscape has diverged significantly. Our results suggest that these evolutionary changes have been mediated through fine-tuning of gene expression programs that are globally conserved among the species. We also developed a framework to systematically elucidate conserved and divergent aspects of human and mouse erythropoiesis.

Results

Conservation and Divergence in Erythroid Signature Gene Expression. We began by interrogating the global gene expression profiles from early, intermediate, and late erythroid precursors from adult humans and mice (15, 16). We first examined genes whose role in erythropoiesis is well studied, including those encoding cytoskeletal proteins, transmembrane proteins, and heme biosynthetic enzymes. The expression values were mean-centered to depict relative changes across terminal erythropoiesis, independently of magnitude (Fig. 1 and Fig. S2). Red blood cell membrane and cytoskeletal proteins make up the components of the filamentous meshwork of proteins along the cytoplasmic surface of the membrane necessary for mechanical stability, normal morphology, and flexibility (22). Dominant mutations in ANKI, SPTA1, SPTB, SLC4A1, EPB42, and EPB41 cause hereditary spherocytosis or elliptocytosis, characterized by poor membrane stability, abnormal morphology, and a high rate of red cell turnover (23, 24). The important function of these proteins implies that their expression must be well conserved among species. Indeed, most of the cytoskeletal proteins show similar patterns of expression in both species. EPB41, TMOD1, SPTA1, EPB42, EPB49, and SPTB increased with maturation in both species, but ADD3 decreased. However, divergence also exists as shown by several genes that are differentially regulated. Human ACTB and TNRFSF1A show no significant changes across the stages, but their mouse orthologs decreased in late stage erythroblasts. Expression of human ANKI and ADD1 showed minor changes with differentiation, but their orthologs progressively increased in mice. Finally, during maturation ADD3 expression increased in humans, but Add3 decreased in mice (Fig. 1A). These findings imply that, although the composition of the red blood cell cytoskeleton is globally conserved in mammals, differences are present in the expression of certain proteins necessary for maintenance of this structural network. These differences may be relevant for our understanding of the human disorders of red blood cell membrane structure, including hereditary spherocytosis and elliptocytosis, and of the mouse models of these diseases (22).

Transmembrane proteins are used to track erythroid cell maturation using flow cytometry, and many compose the clinically relevant blood groups (25, 26). From a limited list (Fig. 1B), ~55% are up-regulated with differentiation in both human and mouse, including GYP A, DARC, and ICAM4. However, CD47, RHAG, TFRC, XK, and CD44 showed divergent expression. CD47, RHAG, TFRC, and XK decreased during human terminal erythropoiesis, whereas their orthologs increased in mouse terminal erythropoiesis; CD44 displayed the opposite pattern (Fig. 1B and Fig. 2A). We confirmed by flow cytometry the differential expression of CD44 protein on human and mouse peripheral red blood cells (Fig. 2B).

Genes for erythroid heme biosynthesis also revealed similarities and differences in their expression patterns. Differentially expressed genes include CP OX, ABCB10, UR O S, URO D, and FECH, and conserved genes include HMBS, ALAS2, SLC25A37, ALAD, PPOX, GLRX5, and ABCB6 (Fig. 1C). This research extends earlier experiments suggesting that the kinetics of heme biosynthesis in human and mouse terminal maturation is different (27).

Fig. 1. Conservation and divergence in erythroid signature gene expression. Mean-centered expression of the average expression values across the early, intermediate-, and late-stage erythroblasts (red/blue color scale to the right) of signature erythroid gene groups: (A) skeletal proteins, (B) transmembrane proteins, (C) heme biosynthesis enzymes, and (D) erythroid-specific transcription factors in humans (Left) and mice (Right). P, proerythroblasts; B, basophilic erythroblasts; PO, mixture of polychromatophilic and orthochromat erythroblasts. ↑, increasing gene expression from P to PO; ↓, decreasing gene expression from P to PO; –, no significant changes from P to PO.

Global Gene Expression Changes During Erythroid Terminal Differentiation. Despite the high conservation of the signature erythroid gene expression patterns, the global gene expression profiles of matched erythroblast stages, i.e., those that compare the expression of all 12,808 orthologous genes, showed a lower degree of conservation. The mean Pearson correlation coefficient between mRNA expression in human and mouse proerythroblasts is 0.66; basophilic erythroblasts, 0.64; and polychromatophilic/orthochromat erythroblasts, 0.67 (Fig. 3A). As essential controls for these interspecies correlations and to confirm that our analyses of these datasets were valid and not skewed by different cell sources, cell purity, and laboratory techniques, we carried out multiple intraspecies and interspecies global gene expression comparisons between these datasets and other publically available datasets.

First, the global gene expression data of the mouse definitive erythroblast cell line, G1E-ER4, which Ga l a1 activity was reactivated with estradiol treatment (29). After 14 and 21 h of estradiol induction, the G1E-ER4 cells differentiate into proerythroblast-like cells. A comparison of the global gene expression profile of these G1E-ER4 cells to mouse primary proerythroblasts yields a strong mean Pearson correlation coefficient of 0.86 (Fig. S3B). At 30 h postinduction, the G1E-ER4 cells resemble basophilic erythroblasts.
and a comparison of the global gene expression profile of these cells and mouse basophilic erythroblasts yields a mean Pearson correlation coefficient of 0.86 (Fig. S3B). For human interspecies comparisons, we compared the datasets in Fig. 3A to those from ex vivo cultured, but unsorted, differentiating erythroid cells from CD34+ hematopoietic stem/progenitor cells (HPSCs) (30). The CD34+ HPSC cells reach a stage resembling proerythroblasts after 5 d and basophilic erythroblasts after 7 d, although quantitation of the purity of these stages was not done in this study. Nonetheless, the mean Pearson correlations with the corresponding proerythroblast and basophilic erythroblast populations are 0.95 and 0.95, respectively (Fig. S3C).

We also carried out interspecies comparisons for all possible dataset combinations to ensure that the results were not biased by one particular dataset and were consistent across all datasets (Fig. S4). We cross-compared the two human datasets to the three mouse datasets (15, 16, 28–30). All interspecies comparisons yielded mean Pearson correlations ranging from 0.832 to 0.993, whereas their interspecies equivalent yielded mean Pearson correlations of only 0.637–0.728 (Fig. S4), consistent with our conclusion that there is a substantial divergence in the transcriptional landscape in the two species independent of the datasets used. Although this analysis is confounded by the use of different probesets among the different microarray platforms, the consistency of these findings implies the reliability of such comparisons to delineate species-specific differences.

To establish that these results were not skewed by lowly expressed genes, we carried out a similar comparison with only the top 500 most highly expressed genes from the human data (Dataset S1); these are the datasets that we used in the initial comparison of the mouse and human erythroblast datasets (Fig. 3B). Surprisingly, there is a much higher degree of divergence with poor correlation between human and mouse proerythroblasts, basophilic erythroblasts, and polychromatophilic/orthochromatophilic erythroblasts, with mean correlations of 0.236, 0.199, and 0.130, respectively (Fig. 3B). These results indicate that there is indeed significant transcriptional divergence between human and mouse erythroblasts, which is most prominent among highly expressed genes. Interspecies comparison for the top 500 most highly expressed genes with all other possible dataset combinations yielded low mean correlations of 0.130–0.329 between the comparable stages (Fig. S5). In contrast, intraspecies comparison yielded mean correlation coefficients between 0.620 and 0.987 (Fig. S5). Once again, the fact that different microarray platforms were used could lead to an underestimation of transcriptional conservation. However, the consistency of the results, as addressed by the various cross-comparisons that we noted above, is in support of the divergence that we observed between human and mouse gene expression. Examining the stark difference in mean correlations between intra- and interspecies comparisons, we can safely deduce that the results are unlikely to be due to technical artifacts. Additionally, we confirmed the consistencies of the gene expression patterns of several erythroid signature genes whose expression pattern differs in humans and mice. We focused on several genes

**Fig. 2.** Differentially regulated genes in human and mouse erythroblasts. (A) CD44 and CD44 gene expressions mined from the comparative gene expression framework. (B) Flow cytometry of the predicted CD44 expression difference by staining human and mouse peripheral blood (isotype controls are shown in red; CD44 antibody staining is in blue). (C) SEC23B, SEC23A, Sec23a, and Sec23b gene expression mined from the comparative gene expression framework. (D) Western blot of Sec23a and Sec23b at all stages of terminal erythropoiesis in mouse fetal liver progenitor cells after 24, 34, and 48 h in culture. ProE, proerythroblasts; Baso, basophilic erythroblasts; PolyO, mixture of polychromatophilic and orthochromatric erythroblasts.
whose expression pattern during terminal erythropoiesis was divergent between humans and mice—ADD1, ADD2, ADD3, CD47, TFRC, ITGA4, CD44, LMO2, and ZFPM1 (Fig. 1)—and showed that all of the expression patterns are in agreement across datasets (Fig. 5).

To further eliminate potential probeset-derived technical complications, we examined the changes in gene expression during the differentiation of proerythroblasts to basophilic erythroblasts (B-P), basophilic erythroblasts to polychromatophilic/orthochromatic erythroblasts (PO-B), and proerythroblasts to polychromatophilic/orthochromatic erythroblasts (PO-P) (Fig. 4). We calculated changes in gene expression between stages using the LIMMA algorithm, yielding the log2 ratio of differential expression between any two stages of differentiation for each orthologous gene (31). We then carried out a global comparison of these gene expression changes to indicate how expression of all 12,808 orthologous genes changes upon differentiation. This method has eliminated the discrepancies due to probeset differences, as the numerical fold changes used in the comparative studies were normalized and calculated within the respective microarray platforms, giving us the “absolute” kinetic trend for each gene. Global comparison of the gene expression changes during terminal differentiation shows significant divergence between humans and mice with a mean correlation of 0.28, 0.22, and 0.32 for B-P, PO-B, and PO-P, respectively (Fig. 4A). A more stringent comparison, involving only those genes that significantly change from one stage to another (adjusted P values < 0.001) in the human data, indicates only slightly better correlation between the two species (Fig. 4B).

Transcriptional Regulation of Human and Mouse Erythropoiesis. Fig. 1D shows that the majority of key transcriptional regulators showed conserved expression patterns during differentiation with few exceptions: TALI and MYB expression exhibited altered temporal regulation. Human TALI is up-regulated only during the polychromatophilic and orthochromatic erythroblast stage, whereas mouse TALI is up-regulated earlier at the basophilic erythroblast stage (Fig. 1D). These differences suggest that subtle changes can dramatically affect the expression of genes regulated by these transcriptional regulatory factors, particularly because combinatorial interactions of several transcription factors and epigenetic regulators can determine the ultimate transcriptional output at any time during differentiation. Strikingly, we found that, although the transcriptional landscape has diverged significantly, predicted transcriptional regulators at each stage—defined by motifs found in the promoters of target genes—suggest that a similar cohort of transcriptional regulators (with ~70% conservation of regulators at the different stages) are required in both species at the same stages (Table S1). These conserved sites included those potentially bound by the canonical regulators of erythroid differentiation GATA1, NF-E2 (represented by AP-1 sites in data analyzed), and KLF1/EKLF (represented by SP1 sites in data analyzed) (32). This is consistent with the notion that specific master transcriptional regulators of differentiation are critical for the process of erythropoiesis in both species (1, 32, 33).

Contribution of Promoter Region to Transcriptional Divergence. To understand the molecular basis for the interspecies divergence in gene regulation during terminal erythropoiesis, we first analyzed the promoter regions of the 749 genes that were in the top 500 expressed genes at any stage of human erythropoiesis analyzed in Figs. 1–4. For simplicity, we defined promoters as the 1-kb region extending from 900 bases upstream of the transcription start site to 100 bases downstream. We calculated the percentage of sequence identity of each of these promoter regions between humans and mice. In parallel, we also analyzed the expression pattern of these genes during terminal differentiation. For each gene, we calculated a Pearson correlation coefficient comparing the expression patterns generated by plotting the mean values of proerythroblasts, basophilic erythroblasts, and mixtures of polychromatophilic and orthochromatic erythroblasts in human and mouse datasets. Strikingly, when promoter regions are highly conserved, defined as a sequence identity of greater than 65%, the expression patterns of these 15 genes are largely consistent between humans and mice, with an average Pearson coefficient of 0.6 (red box in Fig. S7). When there is only modest or low conservation of promoter sequence identity, the expression patterns are more highly variable (Fig. S7). This supports the notion that modifications to proximal promoters contribute to transcriptional divergence, although specific motifs within the promoters may have a more important contribution to the conservation of expression and are difficult to assess systematically on a global scale.

Contribution of Gene Duplication to Transcriptional Divergence. The comparison presented above focuses on one-to-one orthologs, which make up the majority of the human and mouse genome. Nevertheless, gene duplication, gene loss, and de novo formation play a major role in divergence between species (34). To investigate the contribution of such events in the observed transcriptional divergence, we analyzed a specific clinically relevant example. Both SEC23B and SEC23A encode proteins that are critical components of the coat protein complex II (COPII) that is necessary for endoplasmic-reticulum-to-Golgi-complex transport of secretory vesicles (35). In humans, recessive mutations of SEC23B cause congenital dyserythropoietic anemia (CDA) type II (10, 36). Although the exact basis for the pathophysiology of

![Fig. 4.](image)

Global comparison of the gene expression changes during erythroid terminal differentiation in human and mouse. (A) Mean Pearson correlation coefficients (white/blue color scale at the bottom of A and B) comparing the global gene expression changes transitioning from one stage to another during terminal maturation between humans and mice. (B) Mean Pearson correlation coefficients comparing the gene expression changes, including only genes that are significantly changed between stages with adjusted P values < 0.001 in the human datasets and transitioning from one stage to another during terminal differentiation. Gene changes from proerythroblasts to basophilic erythroblasts (B-P); from basophilic erythroblasts to polychromatophilic/orthochromatic erythroblasts (PO-B); from proerythroblasts to polychromatophilic/orthochromatic erythroblasts (PO-P).
this disease is not understood, erythroid cells are thought to be particularly sensitive to mutations in SEC23B due to the lack of expression of SEC23A, which can compensate for the loss of SEC23B in other tissues (10). In contrast, mutations in Sec23b fail to result in erythroid abnormalities in mouse models (11). Using our comparative gene expression framework, we found that Sec23b is upregulated in terminal mouse erythropoiesis, indicating that this paralogous gene may be able to provide compensatory function in the mouse Sec23b knockout, suggesting a potential explanation for the lack of hematologic abnormalities in the Sec23b knockout mouse model (Fig. 2C) (11). To support these findings, we found robust protein expression of Sec23a during all stages of terminal erythropoiesis in mice, whereas Sec23b was greatly reduced at the final stages of differentiation, supporting the concept of compensation in the Sec23b knockout animals (Fig. 2D) and confirming a recently reported finding on the differential expression of Sec23 orthologs related to CDA type II (37).

Discussion

Although specific differences in human and mouse models of erythropoiesis have been identified, to date there has been no systematic comparison of changes in gene expression. Here we developed a framework for comparing global gene expression changes between the two species and generated a resource allowing users to query expression patterns of numerous orthologous genes in humans and mice during terminal erythropoiesis (Datasets S2 and S3). This framework can be used as a reference map to understand and study conservation and divergence between human and mouse erythropoiesis, as well as to serve as a tool for translating findings between species. To expand this resource, similar compendia can be constructed on other species, as well as on a greater range of erythroid cell subsets.

Applying Pearson correlation analyses to the global gene expression data from morphologically identical stage-matched sorted populations of erythroid precursors that represent the distinct stages of terminal erythroid maturation, we find a significant extent of transcriptional divergence between comparable stages in humans and mice. Significant divergence in transcriptional changes occurring between these stages is also observed in the two species. Despite the technical limitations that we have highlighted, including probeset differences and variation in sample preparation, we consistently found substantial divergence of the transcriptional landscape of erythropoiesis between humans and mice using numerous cross-comparisons. Future cross-species comparative studies can be greatly fine-tuned by using improved purification methods and distinct phenotypic markers to obtain highly purified human and mouse erythroblasts at distinct stages, similar to methods recently reported (12). In addition, the use of RNA sequencing data may help eliminate some issues arising from microarray probeset differences that were faced in this analysis. In particular, the datasets that we used did not allow us to analyze differences in gene expression that may be due to or masked by altered gene splicing programs; studies focused on this aspect of gene regulation will undoubtedly help advance our understanding of erythropoiesis even further.

Our results suggest that, although the expression of major red cell proteins and the morphological process of erythropoiesis have remained largely conserved among mammals, a significant divergence of transcriptional regulation has occurred. However, our finding of conservation of transcriptional regulators and their associated genes suggests that a key modification occurring in the course of erythroid evolution was in the responses of multiple target genes to their cognate transcriptional regulators. In particular, and focusing specifically on the top 500 most abundantly expressed genes in human Dataset S1, the interspecies divergence of gene expression patterns was even more prominent. Whereas expression of individual genes can vary significantly between humans and mice, our analysis indicates that, for each stage-matched population, ∼30% of the top 500 genes in human erythroid cells have their orthologous equivalent in the top 500 genes expressed in stage-matched mouse erythroid cells (Dataset S1). Additionally, several metabolic pathways, including iron metabolism and heme biosynthesis, are well known to be shared in mouse and human erythroid cells at the same developmental stage, and this is largely confirmed by our Gene Ontology analysis (Dataset S4). Therefore, the altered expression patterns of many of the genes that comprise these pathways are likely to accommodate the substantial physiological differences between the two species.

Of the top 500 genes expressed in humans and mice at each stage of terminal erythropoiesis, most demonstrate modest amounts of promoter conservation—on the order of 40–60% sequence identity—and there is little correlation between the expression patterns of these genes in humans and mice (Fig. S7). In contrast, high sequence conservation of promoters, as indicated by greater than 65% sequence identity, is associated with similar gene expression patterns in the two species. Clearly, mutations in proximal promoters likely contribute to the interspecies divergence in gene expression, but extensive analyses of binding of multiple transcription factors and chromatin-modifying enzymes to these promoters will be essential to determine precisely how promoter mutations might drive evolutionary transcriptional divergence. Additionally, numerous species-specific properties of distal enhancers likely also contribute significantly to interspecies transcriptional divergence (38). In addition, many noncoding RNAs are expressed in terminal differentiating murine erythroid cells, and at least 12 are essential for red cell formation; additional work is essential to determine whether evolutionary changes in noncoding RNAs also contribute to the divergence in interspecies gene expression patterns that we have uncovered (39, 40).

Given both the similarities and the differences in gene expression in mammalian erythropoiesis, it is difficult to predict a priori whether a particular perturbation in mice may recapitulate what is seen in humans. For example, in mammals there exist two paralogs, Sec23a and Sec23b, with presumably identical functions in COP11 vesicular transport. Transcriptional divergence in these genes might be attributed to gene duplication leading to two paralogous genes with compensatory function, and therefore their expression can diverge significantly between humans and mice. However, Cd44, which is present as a single ortholog in humans and mice, also demonstrates transcriptional divergence despite transcriptional conservation of other membrane proteins expressed on human and mouse erythroid cells. These differences necessitate a systematic framework to understand and study conservation and divergence between human and mouse erythropoiesis. This becomes evident in the case of recessive Sec23b mutations, which in humans results in CDA II. However, the failure of mouse models to recapitulate this disease is most likely attributable to the observed variation in expression of Sec23a and Sec23b in comparison with their human counterparts. Indeed, analyses of published ChIP-seq data show binding of GATA1 to the proximal promoter region of the Sec23a gene in mouse erythroleukemia cells but not in human K562 cells (41, 42). This is only one of several such diseases that fail to be faithfully recapitated by mouse models (2). Thus, developing a compendium of differences derived from global gene expression patterns can help to explain why mouse models fail to mimic specific human diseases and whether studying certain human blood diseases in transgenic mouse models will be appropriate.

This framework can also serve as a predictive tool and a resource to design studies for drug candidates in humans. For example, CDA44 is a critical factor in chronic lymphocytic leukemia disease progression, and the use of antibodies specifically targeting CD44 leads to complete clearance of engrafted leukemic cells in xenograft mouse models (43, 44). However, examining the CD44 expression profile from erythroid gene expression datasets indicates that, unlike in mice where Cd44 expression decreases
upon maturation, human red cells highly express CD44, an observation that we substantiated using flow cytometric analysis of mature red blood cells (Fig. 2B). Therefore, caution needs to be applied to translate such models between species; CD44 antibodies might be readily absorbed by mature human red blood cells in the circulation, decreasing the effectiveness of such treatments, which could not be identified in preclinical xenograft models (43, 44).

This study emphasizes both the value and the limitations of mouse models. Clearly, many aspects of erythropoiesis are conserved, although gene expression has varied considerably in the course of mammalian evolution. By using such a systematic genetic expression framework, logical predictions on the effect of perturbations in human and mouse erythropoiesis can be made and better mouse models of human disease can be rationally designed. In addition, this framework will assist in our understanding of how data on human erythropoiesis derived from primary cell culture, pluripotent stem cells, xenograft models, and human genetics can be merged with the significant amount of information derived from studies of mouse erythropoiesis (2, 3, 45).

Materials and Methods

Microarray data from humans and mice were obtained as discussed in SI Materials and Methods. The data were preprocessed with the GC-RMA algorithm using custom probe set definitions for National Center for Biotechnology Information Entrez Genes derived from the method of Dai et al. (46). Details of the microarray analysis, ortholog mapping, differential gene expression analysis, and experimental approaches (flow cytometry, cell culture, and Western blotting) are provided in SI Materials and Methods.

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