Telomerase is a ribonucleoprotein complex that maintains the length and integrity of telomeres, and thereby enables cellular proliferation. Understanding the regulation of telomerase in hematopoietic cells is relevant to the pathogenesis of leukemia, in which telomerase is constitutively activated, as well as bone marrow failure syndromes that feature telomerase insufficiency. Past studies showing high levels of telomerase in human erythroblasts and a prevalence of anemia in disorders of telomerase insufficiency provide the rationale for investigating telomerase regulation in erythroid cells. Here it is shown for the first time that the telomerase RNA-binding protein dyskerin (encoded by \( \text{DKC1} \)) is dramatically upregulated as human hematopoietic stem and progenitor cells commit to the erythroid lineage, driving an increase in telomerase activity in the presence of limiting amounts of \( \text{TERT} \) mRNA. It is also shown that upregulation of \( \text{DKC1} \) was necessary for expansion of glycophorin A+ erythroblasts and sufficient to extend telomeres in erythroleukemia cells. Chromatin immunoprecipitation and reporter assays implicated GATA1-mediated transcriptional regulation of \( \text{DKC1} \) in the modulation of telomerase in erythroid lineage cells. Together these results describe a novel mechanism of telomerase regulation in erythroid cells which contrasts with mechanisms centered on transcriptional regulation of \( \text{TERT} \) that are known to operate in other cell types. This is the first study to reveal a biological context in which telomerase is upregulated by \( \text{DKC1} \) and to implicate GATA1 in telomerase regulation. The results from this study are relevant to hematopoietic disorders involving \( \text{DKC1} \) mutations, GATA1 deregulation and/or telomerase insufficiency.

**Introduction**

Telomerase is a ribonucleoprotein complex that maintains the length and integrity of chromosomal-end structures called telomeres and thereby enables continuous cellular proliferation. The minimum essential components of the human telomerase holoenzyme are a specialized reverse transcriptase (TERT) and a non-coding RNA (TERC) that includes an RNA template domain for priming synthesis of telomeric repeats. Active human telomerase ribonuclear proteins also include the RNA binding and modifying protein, dyskerin. Dyskerin, encoded by \( \text{DKC1} \), augments telomerase activity by directly binding to TERC to confer the structural rigidity and stability necessary for its accumulation and function. The telomerase enzyme activity underpins the unrestricted proliferation of cancer cells in approximately 80-90% of malignancies, including acute leukemias and lymphomas.
efficient production of glycophorin A-positive (GLYA+) GATA1 and that high expression of DKC1 is required for the functional target of the erythroid-specific transcription factor GATA1. Past studies along these lines have attributed the hematologic conditions associated with insufficient telomerase activity in human hematopoietic cells is a crucial step toward the development of effective treatment of hematologic syndromes and the need for new treatments for these disorders, provided the impetus for investigation in this area.

Here we show that the increase in telomerase activity that occurs as human HSPC commit to the erythroid lineage is a result of upregulation of the DKC1 gene in the presence of limiting amounts of TERT mRNA. It is shown for the first time that the DKC1 gene is a direct transcriptional target of the erythroid-specific transcription factor GATA1 and that high expression of DKC1 is required for efficient production of glycophorin A-positive (GLYA+) erythroblasts. These results provide a novel mechanistic explanation for high levels of telomerase in GLYA+ erythroblasts and the heightened vulnerability of the erythroid compartment to telomerase insufficiency.

Methods

Cord blood cell isolation and culture of CD34+ and glycophorin A+ cells

CB was obtained from the Royal North Shore Hospital and the Australian Cord Blood Bank. Ethical approval for the use of CB was obtained from the Human Research Ethics Committees of the relevant hospitals and the University of New South Wales (approval numbers: HREC 05188, NSCCH 0602-004M, SESIAHS 08/190). Bone marrow mononuclear cells were obtained from Lonza (Mt Waverley Australia). CB processing and isolation of CD34+ HSPC and GLYA+ cells are described in the Online Supplementary Methods. CD34+ HSPC were expanded for 1 week in Isocove modified Dulbecco media (Life Technologies, Carlsbad, CA, USA) with 20% fetal bovine serum (Trace Scientific, Melbourne, Australia), 100 ng/mL stem cell factor (SCF, Amgen, Thousand Oaks, CA, USA), 100 ng/mL thrombopoietin (Peprotech, Rocky Hill, NJ, USA), 100 ng/mL Flt-3 ligand (FIT-3L, Amgen) (STF), 50 µg/mL gentamycin and 200 mM glutamine. The cells were then cultured in cytokine combinations that force expansion and differentiation along specific lineages as described in our previous study (Online Supplementary Table S1). Differentiation was assessed by fluorescence activated cell sorting (FACS) analysis after staining cells with the conjugated antibodies detailed in Online Supplementary Table S2. Green fluorescent protein-positive (GFP+), GLYA+ and CD34+ subpopulations were purified by FACS using a FACS Diva (Becton Dickinson).

DKC1 gene suppression and overexpression

The viral vectors and methods used for suppression and overexpression of DKC1 are described in the Online Supplementary Methods.

Telomerase enzyme assays and telomere length measurements

Telomerase enzyme activity was quantified using the real-time polymerase chain reaction (PCR)-based telomeric amplification protocol (qTRAP) as described elsewhere. Mean telomeric restriction fragment length was measured using the Telomere Length Assay kit (Roche, Mannheim, Germany) as previously described and detailed in the Online Supplementary Methods.

Gene and protein expression analyses

Quantitative real-time PCR (qRT-PCR) and western blot analyses were performed according to standard protocols described in the Online Supplementary Methods.

Chromatin immunoprecipitation and reporter assays

Chromatin immunoprecipitation (ChIP) assays were performed as previously described. Briefly, 2 × 107 cells were treated with 1% formaldehyde, then cross-linked chromatin was sonicated to obtain 300-500 bp fragments. Chromatin was immunoprecipitated with antibodies detailed in Online Supplementary Table S2 and subjected to qRT-PCR using Express SYBR Green (Life Technologies) and the primers described in Online Supplementary Table S3. Values were normalized to products from immunoprecipitation with control IgG antibody.

A DKC1 promoter reporter construct (pGL2-DKCI3L) was made by cloning a DKC1 sequence spanning +211 to -1113 bp from the DKC1 transcription start site into XhoI and HindIII sites of the pGL2 vector encoding luciferase. Two proximal GATA sites were mutated by site-directed mutagenesis using the QuikChange Site-directed Mutagenesis Kit (Stratagen, La Jolla, CA, USA) with the primers listed in Online Supplementary Table S3. The mutated
nucleotides were verified by Sanger sequencing. Luciferase assays were performed using HEL 92.1.7 cells as described in the Online Supplementary Methods.

Statistics
All statistics were performed using GraphPad Prism 6.0b (La Jolla, CA, USA). Results were considered statistically significant when $P<0.05$.

Results

**DKC1 is upregulated with erythroid lineage commitment**

It was previously shown that telomerase activity is upregulated when CB-derived HSPC were switched to conditions promoting erythroid differentiation.14 To verify this finding in a pure population of erythroid cells, GLYA+ cells were sorted by FACS from cultures generated by ex vivo expansion of HSPC. CD34+ cells were first expanded in medium supplemented with STF for 1 week, then switched to medium containing SCF and erythropoietin (SE) for a further 2 weeks. FACS analysis using antibodies for GLYA and CD34 confirmed differentiation of HSPC and enrichment for GLYA+/CD34- erythroid cells (>80% of the viable population) at week 2 and week 3 (Figure 1A and Online Supplementary Figure S1). Erythroid cell populations were further purified from week 2 cultures by FACS sorting cells based on either low or high expression of GLYA and lack of expression of the myeloid cell marker CD13. CD13+/GLYA+ myeloid cells were also purified from the week 2 cultures for comparison with the erythroid cells (Figure 1B). Telomerase activity was quantified in the FACS-sorted populations of GLYA+high, GLYA+low erythroid cells and CD13+ myeloid cells, as well as CD34+ cells and an unpurified population of cells cultured in SE. The results demonstrated that telomerase activity was upregulated in GLYA+high erythroblasts relative to both uncultured CD34+ HSPC and unsorted SE-cultured cells (Figure 1C). In contrast, no significant telomerase activity was detected in CD13+ myeloid cells. These data confirm that telomerase activity was confined to the GLYA+ erythroid subset of cells in the SE culture, and was downregulated in differentiated myeloid cells.

To investigate the regulation of telomerase during erythroid commitment, telomerase enzyme activity and expression of TERT, TERC and DKC1 was assessed at weekly time points over the 3-week culture period. As previously reported, telomerase was modestly upregulated upon initial cytokine stimulation with STF,11,12 then further increased during the second week of culture after switching to SE ($P<0.01$) (Figure 2A).14 In parallel with the initial induction of telomerase activity, there was a measurable increase in TERT expression during the first week.
of culture in STF. TERT expression then returned to the low basal levels detected in unstimulated HSPC when the culture was switched to SE (weeks 2-3). In contrast to TERT, DKC1 expression was induced after cultures were switched to SE. The kinetics of DKC1 upregulation varied among the CB cultures established from different individuals, but invariably increased after the switch to erythroid conditions at week 1 and peaked at either week 2 or 3 of culture (Online Supplementary Figure S2). The DKC1 expression pattern closely paralleled erythroid commitment and expansion, as indicated by expression of GLYA+ (Figure 1A). Expression of TERC did not alter dramatically over the time course, apart from a modest increase from week 1 to 2 of culture.

To determine whether the upregulation of DKC1 expression was specific for the erythroid lineage, DKC1 expression was analyzed in cell populations enriched for monocytic, granulocytic and megakaryocytic cells. Cultures enriched for these lineages were produced by switching STF cultures at week 1 from STF to SCF, FLT-3L.
and monocyte colony-stimulating factor (SFM) for monocytc differentiation, to SCF, FLT-3L and granulocyte colony-stimulating factor (SFG) for granulocytic expansion and to SCF, thrombopoietin and interleukin-6 (ST6) to promote megakaryocyte differentiation (Online Supplementary Figure S2). qRT-PCR analysis showed that in contrast to the upregulation of DKC1 observed in cells cultured with SE (P<0.01), there was no significant induction of DKC1 expression in cells grown under conditions favoring monocytc, granulocytic or megakaryocytic differentiation (Figure 2B). Western blot analysis further demonstrated high expression of dyskerin protein only in cell populations produced under erythroid conditions (Figure 2C).

**Upregulation of DKC1 bolsters telomerase activity and promotes telomere lengthening**

To confirm that DKC1 expression was upregulated in erythroblasts produced in vivo as well as in ex vivo-generated erythroid cultures, DKC1 expression was assessed in GLYA+ cells isolated from uncultured CB and bone marrow mononuclear cells. Results from qRT-PCR analysis consistently showed higher DKC1 mRNA in the GLYA+ fraction than in the GLYA- fraction of cells isolated from four samples (Figure 2D). Consistent with these results, gene expression data, collected through meta-analysis of multiple independent studies (available through the BioGPS Primary Cell Atlas dataset), showed DKC1 expression to be upregulated in proerythroblasts relative to hematopoietic stem cells and myeloid lineage cells (Figure 2E). The upregulation of DKC1 mRNA in bone marrow proerythroblasts appeared to be transient, as DKC1 levels in intermediate erythroblasts were similar to those in hematopoietic stem cells. Collectively these data provide strong evidence of DKC1 upregulation during ery-throid commitment of human HSPC.

To test whether induction of DKC1 was sufficient for the upregulation of telomerase activity observed in erythroblasts, HSPC were transduced with lentiviral vectors encoding DKC1 cDNA plus GFP (MSCV-DKC1) or GFP alone (MSCV-GFP). Robust upregulation of DKC1 mRNA in MSCV-DKC1-transduced cells was demonstrated by qRT-PCR analysis of GFP+ cells isolated by FACS (P<0.01, Student t test) (Figure 3A). Telomerase enzyme activity was substantially increased in MSCV-DKC1 cells relative to control vector-transduced cells (P<0.01, Student t test) (Figure 3B). There were no significant differences in the expression of TERT and TERC between MSCV-DKC1 and control vector-transduced cells (Figure 3A), although TERC expression tended to be higher in the MSCV-DKC1 cells.

To enable analysis of the effect of DKC1 upregulation over a time course sufficient for assessing telomere length changes, we also overexpressed DKC1 in the immortal erythroleukemia cell line, HEL 92.1.7 (Figure 3C). Consistent with the CB experiments, overexpression of DKC1 in HEL 92.1.7 cells caused a substantial elevation of telomerase activity without an apparent change in TERT mRNA expression (Figure 3C, D). Southern blot-based analysis of telomere length showed that ectopic expression of DKC1 resulted in telomere lengthening at a rate of approximately 500 bp over a 2-month period, and 900 bp over 7 months (Figure 3E). To verify that DKC1 upregulation is sufficient for telomere extension, we also overexpressed DKC1 in HL-60 cells. Consistent results were obtained, showing that DKC1 overexpression resulted in robust upregulation of telomerase and elongated telomeres (Online Supplementary Figure S4). These data show that upregulation of DKC1 results in an accumulation of functional telomerase complexes capable of telomere elongation.

**High expression of DKC1 is necessary for erythroblast proliferation**

We next tested whether elevated expression of DKC1 was necessary for erythroid cell proliferation and differentiation. For these investigations CB-derived HSPC were transduced with retroviral vectors encoding one of two different shRNA targeting DKC1 mRNA (D2 and D3) or a non-silencing shRNA (NS) plus GFP. HSPC were pre-stimulated and transduced in medium supplemented with STF and then FAC-sorted for GFP+ cells, which were then cultured in SE. qRT-PCR analysis confirmed effective suppression of DKC1 and corresponding downregulation of telomerase activity in erythroid cells transduced with D2 and D3 vectors relative to control vector-transduced cells (Figure 4A, B). The suppression of telomerase activity was not attributable to reduced expression of TERT, which was expressed at equivalent levels in D2-, D3- and NS-transduced cells (Figure 4C). TERC levels varied among the independent experiments, although they tended to be lower in D2- and D3-transduced cells relative to control cells (Figure 4D), consistent with the known role of dyskerin as a stabilizing scaffold for TERC.

Weekly counts of transduced cells revealed that shRNA-mediated suppression of DKC1 expression inhibited proliferation in SE-driven cultures (Figure 4E). Since there was no apparent difference in the proportion of GLYA+ cells in D2 and D3 cultures compared to NS cultures, the reduction in GLYA+ cell number did not appear to be due to impaired erythroid differentiation (Figure 4F). When plated in methylcellulose, D2- and D3-transduced cells formed erythroid colonies with normal burst-forming unit-erythroid (BFU-E) size and morphology; however, significantly fewer colonies were generated by D2 and D3 cultures than by NS (Figure 4G). In contrast to the effect of DKC1 knockdown on BFU-E colony numbers, there was no discernible effect on colony-forming units of granulocyte-monocyte or granulocyte-erythrocyte-megakaryocyte. Together, the results demonstrate a critical role for DKC1 expression in erythroid proliferation that is independent of differentiation.

**GATA1 contributes to transcriptional regulation of DKC1 in erythroblasts**

The DKC1 promoter was previously shown to be a target of MYC family oncoproteins in MYC-driven cancers. Since MYC is also expressed in erythroid progenitors, we next investigated whether MYC or other erythroid transcription factors, namely GATA1 and TAL1, play a role in the regulation of DKC1. Western blot analysis of uncultured CB CD34+ HSPC and ex vivo-expanded CB cells revealed MYC expression in unstimulated CB HSPC and in cells harvested from STF, STM and SE cultures (Figure 5A). In contrast, expression of GATA1 and TAL1 was confined to cells cultured in SE. Consistent with the qRT-PCR results (Figure 2B), dyskerin protein was detectable in undifferentiated cells cultured in STF and erythroid cells at weeks 2 and 3, but was not detected in cells from monocyte-enriched cultures (Figure 5A).

Canonical E-boxes have previously been identified in
the DKC1 promoter.\textsuperscript{26,27} ChIP sequencing data from peripheral blood erythroblasts made available through ENCODE on the UCSC browser also provide evidence of GATA 1 binding at the DKC1 promoter, in the vicinity of -1097 to -493 relative to the transcription start site (chrX: 153,991,030, hg19) (Figure 5B). Guided by these data, we identified putative GATA sites at positions: -679 to -668 and -453 to -468 and designed PCR primers to interrogate the transcription factor binding by ChIP. First, using antibodies to trimethylated H3K4 (H3K4me3), which occupies transcriptionally active chromatin, and H3K27me3, which identifies repressed sites, we verified that chromatin at the DKC1 promoter was in an open configuration conducive to transcriptional activation in both STF-stimulated cells and erythroblastic cells (Figure 5C).\textsuperscript{29} ChIP analysis also confirmed MYC binding to the DKC1 promoter, although this appeared to diminish progressively as undifferentiated CB cells underwent erythroid differentiation in SE culture. Conversely, GATA1 binding at the DKC1 promoter appeared most robust at week 3, corresponding with the accumulation of erythroblasts expressing high levels of GLYA (Online Supplementary Figure S5) and peak expression of DKC1 in six out of nine CB cultures (Online Supplementary Figure S2). Consistent with GATA1 binding in GLYA\textsuperscript{hi} CB-derived erythroblasts (Figure 5C), ChIP sequencing results from three independent investigations, accessed using CistromeDB, showed GATA1 binding at the DKC1 promoter of erythroblasts derived from bone marrow and peripheral blood (Online Supplementary Figure S6).\textsuperscript{30-32} Although TAL1 can bind DNA via E-boxes, no TAL1 binding at the DKC1 promoter was detected in CB cells at any stage of culture. Collectively, these data suggest a model whereby MYC binds the DKC1 promoter in undifferentiated cells and is replaced by GATA1 during erythroid differentiation.

Since GATA1 regulation of DKC1 has not previously been described, a luciferase reporter assay was conducted to confirm that GATA sites contribute to DKC1 transcription. Mutations were induced in two potential GATA1 binding sites of the DKC1 promoter construct by site-directed mutagenesis and luciferase activity was measured.
in transfected HEL 92.1.7 erythroleukemia cells. The results demonstrated that both GATA sites contributed to promoter activity, and that concurrent ablation of the two GATA sites substantially diminished promoter activity (Figure 5D). Collectively the results provide strong evidence that the erythroid-restricted transcription factor GATA1 contributes to the regulation of DKC1.

Discussion

This report is the first to implicate GATA1 in the regulation of telomerase and to describe a biological context in which telomerase activity is upregulated by induction of DKC1. Here it is shown that GATA1 binds the DKC1 promoter and contributes to the upregulation of DKC1.

Figure 4. Downregulation of DKC1 expression suppresses telomerase activity and inhibits erythroid cell proliferation but not differentiation. Cord blood-derived CD34+ hematopoietic stem and progenitor cells were transduced with retroviral vectors encoding DKC1-targeted shRNA (D2 and D3) or non-silencing RNA (NS) and green fluorescent protein (GFP). GFP+ cells were sorted by fluorescent activated cell sorting (FACS) (week 1), then cultured with stem cell factor plus erythropoietin (SF) for a further 2 weeks. Expression of telomerase genes and telomerase enzyme activity were measured by quantitative real-time polymerase chain reaction and the qTRAP assay, respectively. (A) DKC1 mRNA, (B) telomerase enzyme activity, (C) TERT mRNA and (D) TERC abundance. (E) Expansion of FACS-sorted GFP+ cells determined from cell counts using trypan blue exclusion of dead cells. (F) Percentage of glycophorin A-positive cells in cultures determined by weekly FACS analysis. (G) Erythroid progenitors in the GFP+ sorted fraction were quantified as burst-forming units-erythroid in methylcellulose assays. Values are means ± standard error of mean from six independent experiments. *P<0.05, **P<0.01, ***P<0.001 from the Dunnett multiple comparison of D2 and D3 to NS. AU: arbitrary units; BFU-E: burst-forming unit-erythroid; GLYA+: glycophorin A-positive cells.
which drives telomerase activity levels in erythroid cells. These results are notable in relation to past studies that established the paradigm of TERT transcriptional regulation as the primary determinant of telomerase regulation in hematopoietic and cancer cells. Past investigations of telomerase regulation in hematopoietic cells focused on lymphoid cells, myeloid progenitors and myeloid leukemia cell lines. These studies revealed that mitogen-induced upregulation of telomerase was followed by telomerase downregulation during differentiation. Studies of lymphoid and myeloid cells also established a direct role for MYC in the transcriptional regulation of TERT and telomerase re-activation in hematopoietic cells. Consistent with this paradigm, the present study found parallel upregulation of TERT and telomerase activity in the presence of MYC protein in CB progenitor cells stimulated with STF. However, there was a clear dissociation of this pathway when cultures were switched to erythroid conditions. Upon switching cultures from STF to SE, the abundance of MYC protein was sustained, but TERT expression declined to an apparently rate-limiting level while telomerase enzyme activity escalated. Rather than correlating with TERT expression, the increase in telomerase activity detected in erythroblastic cells correlated with upregulation of endogenous DKC1 mRNA. Modulation of DKC1 expression by overexpression or targeting with shRNA confirmed that DKC1 regulated telomerase activity in erythroid cells without altering TERT gene expression. The functional significance of these observations was further supported by evidence of telomere lengthening following upregulation of DKC1 in the HEL 92.1.7 erythroid cell line. DKC1 mRNA was shown to be abundant in CB-derived CD34+/GLYA+ erythroid cells irrespective of

![Figure 5. GATA1 interaction and regulation of the DKC1 promoter in erythroid cells.](image_url)

(A) Immunoblot of nuclear extracts showing expression of dyskerin and the transcription factors MYC, GATA1, and TAL1 in cord blood (CB) cells cultured for 1 week in medium supplemented with stem cell factor (SCF), thrombopoietin and Flt-3 ligand, followed by culture with SCF plus erythropoietin or SCF, Flt-3 ligand and monocyte colony-stimulating factor. The erythroleukemia cell line TF-1 was used as a control for dyskerin expression. The immunoblot was hybridized to Lamin B1 antibody as a loading control. The image shows samples run on a single gel, with consistent exposure for all samples. The gap between lanes 4 and 5 represents deletion of a failed sample. (B) Schematic figure of the DKC1 promoter showing the region included in the pGL2-DKC1L reporter construct, as well as canonical GATA motifs (indicated by arrows labeled A and B), and regions of GATA1 and MYC binding in peripheral blood erythroblasts and other cell types as reported in ENCODE chromatin immunoprecipitation sequencing traces (UCSC browser GRCh37/hg19). (C) Chromatin immunoprecipitation was performed on ex vivo-expanded CB cells using antibodies to MYC, GATA1, TAL1 and trimethylated histones or a control IgG antibody. Transcription factor binding was quantified as fold-enrichment of quantitative real-time polymerase chain reaction (qRT-PCR) products amplified from the region of interest relative to control region in an unrelated gene. Data were normalized to results from the IgG control antibody. *P<0.05, **P<0.01, analysis of variance followed by the Bonferroni multiple comparisons test. (D) Site-directed mutagenesis was performed to introduce point mutations at canonical GATA sites within a pGL2-DKC1L luciferase reporter construct. HEL 92.1.7 cells were co-transfected with the reporter constructs and control vector pEFBOS-LacZ for normalization. Promoter activity was detected as luciferase activity and measured 48 h after transfection. Values are means ± standard error of mean from three independent experiments. *P<0.05, **P<0.01, ns, not significant, from the Dunnett multiple comparisons test. STF: stem cell factor, thrombopoietin and Flt-3 ligand; SE: stem cell factor plus erythropoietin; SFM: stem cell factor, Flt-3 ligand and monocyte colony-stimulating factor.
whether they were generated ex vivo or in vivo. Consistent with these findings, publicly available microarray data from fractionated bone marrow showed high levels of **DKC1** expression in proerythroblasts.

In addition to regulating **TERT**, MYC has been shown to bind and activate the **DKC1** promoter in tumor cell lines.26,27 The present study adds to this knowledge by demonstrating that MYC binds the **DKC1** promoter in primary human hematopoietic cells. Notably however, MYC binding at the **DKC1** promoter appeared to decline, while **DKC1** was upregulated during erythroid commitment and expansion. These results suggest that MYC plays a less prominent role in driving **DKC1** expression in erythroblasts compared with undifferentiated HSPC. Instead, our study highlights a potential role for GATA1 in the regulation of **DKC1** in the erythroid lineage. This was evidenced by enrichment of GATA1 at the **DKC1** promoter in GLYA erythroblasts, and an apparent transcriptional requirement for GATA binding sites in the proximal region of the **DKC1** promoter.

Consistent with the known role of dyskerin in stabilizing **TERC**38 we consistently observed an increase in **DKC1** promoter activity through direct interactions with H/ACA box RNA-binding proteins.27 The implications of dyskerin’s function in ribosome biogenesis and dyskeratosis congenita are also primary characteristics of the bone marrow failure disorders referred to as ribosomopathies, which feature impaired ribosome biogenesis as an underlying cause.39 Ribosome dysfunction in these disorders is usually attributed to mutations in genes with known roles in ribosome biogenesis. However, the discovery of GATA1 mutations in the ribosomopathy Diamond Blackfan anemia raises the possibility that dyskerin insufficiency may contribute to the pathogenesis of this genetic subtype.26

Collectively, the results from these investigations reveal a novel mechanism of telomerase regulation in primary human erythroblasts which contrasts with the established paradigm centered on MYC-mediated regulation of **TERT** expression in HSPC, lymphocytes and myelomonocytic cells. Notwithstanding the requirement for a rate-limiting amount of TERT for telomerase activity,52 this study shows that **DKC1** expression levels are a critical determinant of telomerase enzyme levels in proliferating erythroblasts. Evidence provided herein that GATA1 contributes to the regulation of **DKC1** has implications in hematopoietic disorders that feature **DKC1** mutations, GATA1 deregulation and/or telomerase insufficiency.

### Acknowledgments

We thank midwives, mothers and neonates at Royal North Shore Hospital and Royal Hospital for Women for cord blood donations to this project. We also thank Prof Emyry Bresnick from University of Wisconsin School of Medicine and Public Health for the GATA1 antibody, and Prof Inderjeet Dokal, Queen Mary University of London, for the pCL10.1-DKC1 lentiviral vector. This project was funded by the National Health and Medical Research Council (107911, 510378, RG150480, RG170246), Cancer Council NSW (RG08-03), Cancer Institute NSW and the Anthony Rothe Memorial Fund.

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