HHS Public Access

Author manuscript

Expert Rev Hematol. Author manuscript; available in PMC 2018 August 24.

Published in final edited form as:

Expert Rev Hematol. 2018 March ; 11(3): 169–184. doi:10.1080/17474086.2018.1436965.

GATA1 insufficiencies in primary myelofibrosis and other hematopoietic disorders: consequences for therapy

Te Ling[1], **John D. Crispino**[1], **Maria Zingariello**[2], **Fabrizio Martelli**[3], and **Anna Rita Migliaccio**[4,5]

[1]Division of Hematology/Oncology, Northwestern University, Chicago, IL, USA

[2]Department of Medicine, Campus Biomedico, Rome, Italy

[3]National Center for Drug Research and Evaluation, Istituto Superiore di Sanità, Roma, Italy

[4]Department of Biomedical and Neuromotorial Sciences, Alma Mater University, Bologna, Italy

[5]Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai (ISMMS), New York, NY, USA

Abstract

Introduction: GATA1, the founding member of a family of transcription factors, plays important roles in the development of hematopoietic cells of several lineages. Although loss of GATA1 has been known to impair hematopoiesis in animal models for nearly 25 years, the link between GATA1 defects and human blood diseases has only recently been realized.

Areas covered: Here the current understanding of the functions of GATA1 in normal hematopoiesis and how it is altered in disease is reviewed. GATA1 is indispensable mainly for erythroid and megakaryocyte differentiation. In erythroid cells, GATA1 regulates early stages of differentiation, and its deficiency results in apoptosis. In megakaryocytes, GATA1 controls terminal maturation and its deficiency induces proliferation. GATA1 alterations are often found in diseases involving these two lineages, such as congenital erythroid and/or megakaryocyte deficiencies, including Diamond Blackfan Anemia (DBA), and acquired neoplasms, such as acute megakaryocytic leukemia (AMKL) and the myeloproliferative neoplasms (MPNs).

Expert Commentary: Since the first discovery of *GATA1* mutations in AMKL, the number of diseases that are associated with impaired GATA1 function has increased to include DBA and MPNs. With respect to the latter, we are only just now appreciating the link between enhanced JAK/STAT signaling, GATA1 deficiency and disease pathogenesis.

Keywords

GATA1; erythropoiesis; congenital anemias; ribosome deficiencies; myeloprol myeloproliferative disorders; myelofibrosis

Corresponding author:Anna Rita Migliaccio,Department of Medicine,Mount Sinai School of Medicine,One Gustave L Levy Place, box #1079,New York, NY 10029, USA. annarita.migliaccio@mssm.edu.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

1. Introduction

GATA1 is the founding member of a family of transcription factors which bind the consensus sequence GATA (hence their name) present in the regulatory regions of all the erythroid-specific genes including the erythropoietin (EPO) receptor *(EPO-R)* and *Gata1* itself [1,2]. The members of this family are characterized by a highly conserved tertiary structure which include an NH- terminal domain, that may establish association with the retinoblastoma protein [3], two DNA-binding zinc finger domains, the NH-terminal zinc finger which binds DNA with the transcription factor friend of GATA1 (FOG-1) as obligatory partner, and the COOH-terminal zinc finger which binds DNA directly and whose binding activity is inhibited by association with the myeloid-specific purine-rich binding box 1 (PU-1) transcription factor. This last observation has suggested that the relative concentration of GATA1 and PU-1 may represent the switch which commits multilineage progenitor cells toward the erythro-megakaryocyte (high GATA1) or granulo-monocyte (high PU-1) lineage. GATA1 has also a COOH-terminal domain whose functions have not been fully established. In addition to recruiting other transcription factor complexes that directly activate gene expression, GATA1 may also recruit epigenetic regulator complexes that regulate chromatin conformation.

GATA1 is expressed within hematopoietic cells at multiple levels. First, it is already expressed, although at very low levels, in hematopoietic stem cells [4,5]. Its expression then increases during the transition from stem cells to the common myeloid progenitor cells (CMP). Lineage restriction is associated with a further increase in GATA1 expression in megakaryocyte-erythroid progenitor cells (MEP) and by a decrease in expression in granulo-monocytic progenitor cells (GMP). Among differentiated cells, high levels of GATA1 expression are detected in erythroblasts and megakaryocytes while the expression of this mRNA is low in eosinophils, mast cells [6–8] and dendritic cells [9,10]. Of note, T and B cells do not express *GATA1* mRNA. Among non-hematopoietic cells, GATA1 expression is restricted to the Sertoli cells of the testis [10–12].

Loss and gain of function studies in mice have demonstrated that expression of GATA1 is absolutely required only for the development of erythroid cells [13]. In fact, in mice, embryos lacking this gene die between day 10.5 and 11.5 (E10.5-E11.5) from severe anemia. Erythroid cells within these embryos are arrested at an early proerythroblast-like stage [14] and die of apoptosis. By contrast, unlike erythroid cells, GATA1 deficiency does not blocks megakaryocyte production but instead induces increased proliferation while delaying maturation [12,14]. Parallel experiments have demonstrated that forced expression of GATA1 in mice activates the expression both of erythroid and megakaryocytic genes [15–17].

The hypothesis that GATA1 specifically regulates the differentiation of erythroid and megakaryocytic cells is also supported by numerous clinical observations indicating that mutations which impair the DNA binding affinity of either the NH-terminal or the COOH-terminal zinc finger of GATA1 are associated with X-linked hereditary disorders expressing an erythroid and/or megakaryocyte phenotype (both in mice and men *GATA1* is localized on

the X chromosome) [18]. These mutations affect the binding of the essential cofactor Friend of GATA1 (FOG-1), impair binding to DNA and/or interfere with the recruitment of the stem cell leukemia (SCL) cofactor [19].

Distinct *GATA1* mutations are seen in two seemingly unrelated disorders, Diamond Blackfan Anemia (DBA) and Acute Megakaryoblastic Leukemia in children with Down syndrome (DS-AMKL) [1]. This class of mutations results in exclusive production of a NH-terminally deleted isoform of GATA1 known as GATA1s [20]. With respect to the DBA phenotype, studies have shown that Gata1s fails to occupy chromatin of erythroid target genes to the same extent as the full-length protein, likely explaining the erythroid defect [21,22]. By contrast, the mechanism by which GATA1s causing mutations contribute to DS-AMKL is unknown.

Recent studies have found an association between the ribosome and GATA1. For example, Sankaran and colleagues demonstrated that the protein, but not mRNA, levels of GATA1 are vastly and selectively reduced in erythroblasts of patients with DBA with ribosomal gene mutations [23]. This reduction appears to be due to the unique 5' cap structure of the *GATA1* mRNA, which makes its translation very sensitive to the levels of functional ribosomes. Another study has shown that the reduced levels of GATA1 observed in megakaryocytes from patients with the MPN subtype primary myelofibrosis (PMF) is likely due to a ribosomal deficiency induced by activated Janus kinase (JAK)/Signal transducer and activator of transcription (STAT) signaling [24]. Bodine and colleagues reported the failure to upregulate components of translational apparatus in DBA with *GATA1* mutations based on RNA sequencing data analysis [25]. Together analysis of patients with defects in erythroid cells and/or megakaryocytes has taught us that mutations or impaired expression of *GATA1* is a contributing factor to a subset of these diseases. In this review, we summarize the current state of understanding of the contributions of GATA1 to normal and defective hematopoiesis.

2. GATA1 regulates transcription in conjunction with multiple cofactors

Many elegant studies have demonstrated that GATA1 exerts classical transcription factor activity, i.e. it binds specific cis-elements in the enhancer and promoter regions of erythroid and megakaryocyte genes and promotes recruitment of the polymerase complex [18]. Subsequent studies however have demonstrated that GATA1 exerts these functions in combinations with other proteins in complexes which may either promote or inhibit gene transcription depending on the context [26]. It has also been reported that the effects exerted by GATA1 on gene transcription depend on its concentration relative to another member of the GATA family, GATA2 [27]. For example, in the case of stem and progenitor cells, the level of GATA2 is far greater than that of GATA1, which tips the balance towards proliferation. Meanwhile, progression from the MEP to erythroid and megakaryocyte precursor cells is associated with a reduction in GATA2 expression and a concomitant increase in GATA1, which in turn promotes maturation. These relative changes in protein concentrations are controlled in large part by the "GATA switch," which plays a critical role in determining the cellular output of the differentiation process [28]. This switch was hypothesized for the first time by Bresnick and colleagues who showed that GATA1

displaces GATA2 at an upstream region of the ~70 kb element of the *Gata2* locus, leading to repression of the gene in erythroid precursors [29].

Since high levels of GATA2 maintain the proliferation of progenitor cells, it is not surprising that alterations in the GATA switch are observed in hematopoietic disorders associated with aplasia (e.g. an accelerated switch in myelodysplastic syndromes) [30] and disorders with increased proliferation (e.g. a delayed switch in Philadelphia-negative MPN). It is important to note that GATA1s, the only GATA1 isoform found in DS-AMKL and some cases of DBA, is less able to repress *GATA2* expression [31,32]. Therefore, a defect in the GATA switch may be a major component of these disorders.

3. GATA1 associates with several epigenetic regulators

Elegant studies by the Blobel laboratory demonstrated that during cytokinesis, erythroid cells shed the majority of chromatin bound transcription factors [33,34]. One of the few exceptions is GATA1, which remains bound to chromatin from one cell generation to the next. This observation, referred to as “bookmarking”, suggests that GATA1 is poised to recruit essential cofactors in the G1 phase of the cell cycle and rapidly reactivate key genes. This phenomenon also likely facilitates recruitment of functional epigenetic complexes: A number of studies have identified GATA1 as a partner of multiple epigenetic modifiers such as NuRD (nucleosome remodeling and deacetylase) and PRC2 (polycomb repressive complex 2)[35–37].

Several lines of evidence show that GATA1 promotes changes in chromatin configuration. First, there is a strong association between sites occupied by GATA1 and those subjected to epigenetic changes: Hardison and colleagues found that, during erythroid development, GATA1 occupied chromatin regions with high level of H3K4me1 (a marker for active enhancers) and low level of H3K27me3 (a marker of repressed promoters) around the canonical GATA binding motif [38]. In addition, Strouboulis and colleagues found that sequences enriched in H3K27ac (another marker of active enhancers) were predominantly bound by GATA1 in fetal liver cells [39]. These results suggested that in addition to binding transcription start sites, GATA1 promotes the transcription of its target genes also by binding and activating their enhancers.

Similar to other transcription factors that lack catalytic activity, GATA1 collaborates with many epigenetic complexes to fulfill its duties as a transcriptional regulator. One way it does this is through its partner FOG-1. FOG-1 binds to the histone deacetylase containing nucleosome remodeling and histone deacetylase (NuRD) complex via its NH-terminal motif [40]. NuRD is generally considered as a corepressor of GATA1-mediated gene regulation. However, the tripartite complex GATA1/FOG-1/NuRD has been shown to activate and repress GATA1/FOG- 1 target genes. What makes NuRD have opposite functions on different gene contexts? In one study, Qiu and colleagues showed that the deacetylase activity of GATA1-associated NuRD complex decreases, committing the complex to become an activator, during erythroid differentiation [41].

PRC2 contains multiple subunits i.e., suppressor of zest 12 homolog (SUZ12), empty envelop deposit (EED), enhancer of zest homolog 1 (EZH1) and EZH2, and is involved in various biological processes. PRC2 has H3K27 methyltransferase activity through its enzymatic components EZH1 and EZH2 [42]. GATA1 has been found to physically interact with PRC2 (SUZ12 and EZH2), and via this complex, H3K27me3 was enriched around the transcriptional start sites of genes with low or no gene expression in the G1E-ER4 erythroid cell line [36,38]. Furthermore, disruption of PRC2 function through EED knock out blocked erythroid maturation in mice [43,44].

Orkin and colleagues recently reported that the GATA switch regulates a switch between EZH1 and EZH2 during erythropoiesis. This latter switch appears to be mediated by occupancy of GATA1 and TAL1 with the +46 (kb) erythroid-selective enhancer of *EZH1* in proerythroblasts. Of note, this occupancy is not detected in hematopoietic stem cells (HSC). Interestingly, another genomic region of *EZH1,* +38 (kb), is strongly occupied by GATA2 in HSC [37], and deletion of this element leads to upregulation of *EZH1* mRNA in erythroid cells. By contrast, disruption of the +46 enhancer failed to activate *EZH1* expression in erythroid cells upon differentiation. It is interesting to speculate that alterations in the GATA1 switch contribute to leukemic transformation of HSC (see later) by altering the EZH switch.

4. Cytoplasmic functions of GATA1

In addition to nuclear complexes, GATA1 may be associated with cytoplasmic complexes which fine tune the concentration of active proteins, as well as the concentrations of other transcription factors, in response to external stimuli. As an example, stem cell factor (SCF) induces ERK (extracellular signal-related kinase) phosphorylation which results in the formation of the NuRSERY (nuclear remodeling shuttle erythroid), complex that is responsible to shuttle phosphorylated ERK, HDAC5 (histone deacetylase 5), GATA1 and KLF1 (kruppel- like factor 1) to the nucleus [45]. We hypothesized that this complex might favor erythroid maturation by balancing the levels of GATA1 and KLF1, another erythroid-specific transcription factor required for terminal maturation, in the nucleus. This hypothesis was recently confirmed by Han et al. who demonstrated that loss of function of cyclin p19, an element downstream of c-KIT, the receptor for SCF, reduces the number and maturation state of erythroblasts genera by $CD34^+$ cells. The effects of Cyclin p19 on erythroid expansion are exerted through a phospho-ERK dependent mechanism and are mediated by reductions in GATA1 content and increased apoptotic rates [46].

5. Human GATA1: one gene but two isoforms partially different functions

The human *GATA1* gene encodes two protein isoforms: GATA1 full-length and GATA1-short (GATA1s). GATA1s lacks the first 83 amino acids that contains the transactivation domain of this transcription factor and may result from both alternative splicing and alternative translation [20,47,48].

Although it has become clear that the absence of full length GATA1, or just the overexpression of GATA1s provides a proliferative advantage to megakaryocyte progenitors

[31], the requirement for GATA1s in normal hematopoiesis is unknown. Comparison of chromatin binding signatures of GATA1 and GATA1s revealed that while GATA1s bound megakaryocytic genes normally, it bound many of its erythroid targets less efficiently than full-length GATA1 [21,22].

GATA1 truncating mutations have been detected in the vast majority of individuals with DS-AMKL. However, the *GATA1s* mutation was not found in leukemic cells of patients with DS who had other types of leukemia or in patients with AMKL without DS [49]. *GATA1* mutations are also associated with transient myeloproliferative disorder (TDM), a self-limiting preleukemia that is prevalent in newborns with DS [50]. To better understand GATA1s and its relationship with DS-AMKL and TMD, Orkin and colleagues generated mice expressing GATA1s in place of the full-length protein [31]. Although GATA1s perturbs fetal hematopoiesis and leads to hyperproliferation of yolk sac and fetal liver progenitors, the adult mice were not found to have overt hematopoietic defects. Of note, expression of GATA1s in fetal liver or ES cells resulted in hyperproliferation of megakaryocytic progenitors [31]. Expression of GATA1s in GATA1-deficient hematopoietic cells also led to megakaryocyte expansion with impaired differentiation [51,52]. GATA1s was also observed to enhance megakaryopoiesis in the context of human induced pluripotent stem cells (iPSCs) with a *GATA1* truncating mutation [22,51]. Germ line *Gata1s* truncating mutations have been found in some individuals without DS [48,53]. In these cases, the *GATA1* mutations do not result in TMD or AMKL, but rather impaired erythropoiesis. With respect to DBA, Sankaran and colleagues discovered *GATA1* mutations at the first translation initiation codon or at the site of splicing of exons 2 and 3 which block production of the full-length protein [23,48]. Hollanda and colleagues had previously reported a family with GATA1s mutations that present with macrocytic anemia [53]. Together these observations indicate that expression of full length GATA1 is necessary for proper development of megakaryocytes and erythroid cells.

6. The mechanism(s) underlying erythroid-megakaryocyte specific GATA1 functions: when concentration matters

The strong structural similarity among proteins of the GATA family makes the strict requirement for GATA1 in erythroid and megakaryocyte lineage development surprising. To explain this paradox, it was hypothesized that, by contrast with other transcription factors, the lineage specific functions of the GATA family members depend on a tight regulation of their expression rather than on their protein structure. This hypothesis was first tested by the Philipsen laboratory, which demonstrated that the embryonically lethal phenotype induced by the *Gata1*^{null} mutation in mice is rescued by forced expression of either *Gata1, 2 or 3* provided that they are expressed in the appropriate spatiotemporal pattern [54]. This hypothesis was further tested by additional studies that aimed to identify the lineage-specific enhancers of *Gata1.* Proof for the existence of these regions was first provided by Nicolis et al. [55] The regions were then refined by additional studies by the Yamamoto and Orkin laboratories which pioneered the use of knock-down mutations, i.e. deletion of regulatory regions which reduce mRNA translation, to identify lineage specific enhancers. These studies revealed that *Gata1* contains three main hypersensitive sites which were marked as

hypersensitive site (HS) HS1, 2 and 3, with HS1 being the enhancer that primarily drives erythroid and megakaryocytic-specific expression [56–58].

By contrast to a complete loss of *Gata1* (*Gata1*^{nu11}), which is embryonic lethal [14], hypomorphic mutations induce a less severe phenotype that allows for the investigation of the effects exerted by alterations in GATA1 levels in adult hematopoiesis. The two most studied hypomorphic mutations are represented by an extensive deletion of the regulatory regions which reduce the *Gata1* mRNA level down to 5% in most cells, the *Gata1*$^{.05}$ mutation [59], and deletion of HS1 only, also known as *Gata1*^{low} mutation, which was originally reported to reduce gene transcription down to 20% [60]. However, more detailed analyses of the levels of *Gata1* expression in cell populations prospectively isolated from *Gatl1*^{low} mice indicated that this mutation reduces the levels of *Gata1* mRNA in a cell-type specific fashion: the levels of *Gata1* mRNA were reduced by 10-fold in CMP, 60% in MEP/pro-erythroblasts and 100-fold in orthochromatic erythroblasts (**Table 1**).

Both *Gata1*$^{.05}$ and *Gata1*^{low} males die of profound anemia at birth. By contrast, heterozygote.females survive to adulthood, but eventually develop hematopoietic neoplasms. *Gata1*$^{.05/+}$ females develop a fatal transplantable leukemia with expansion of the erythroid committed progenitor cell population with the phenotype c-KIT^{+}/CD71^{+} [61]. *Gata1*$^{low/+}$ females instead develop a phenotype very similar to that of the myeloproliferative neoplasm myelofibrosis at 10 months of age [62]. This disorder is associated with expansion of an abnormal MEP population capable at the single cell level to undergo spontaneous immortalization in culture [63]. Of interest, although CMP and MEP from *Gata1*^{low} mice also express reduced levels of *Gata2,* overall the *Gata1/Gata2* mRNA ratio in CMP and MEP from these mutant mice is lower than in their wild-type littermates (0.1 and 0.6 *vs.* 0.75 and 1.3, **Table 1**). This observation was the first indication that delays in the GATA switch may predispose to neoplastic transformations. This was independently tested by the Weiss laboratory, who then demonstrated that hypomorphic *Gata1* mutations induce murine ESC to generate growth-factor dependent erythroid cell lines [64].

Both the *Gata1*$^{0.5}$ and *Gata1*^{low} mutations induce neoplastic transformation in the C57BL/6 but not in the DBA strain [61,65,66]. Of note, hemizygous *Gata1*^{low} mice carrying the mutation in the CD1 background do not die at birth but recover from their anemia at 1 month of age by developing extramedullary hematopoiesis in spleen. The great erythropoietic activity of the spleen from these mutants was confirmed by the observations that removal of the spleen resulted in death of the animals by anemia within 15 days [67].

7. Different mechanisms regulate recovery from acute and chronic erythroid stress in mice

The fact that in the CD1 background *Gata1*^{low} mice have an apparently normal life span and survive until 20–22 months of age [66] allows for investigations on the effects exerted by long-term reduction of *Gata1* expression on hematopoiesis. The blood values of *Gata1*^{low} and wildtype littermates at 5–6 months of age are described in **Table 2.** Although non-anemic, the mutant mice express reduced levels of many erythroid values (erythrocyte numbers, hemoglobin and hematocrit) while the mean corpuscular volume of the red blood

cells is greater than normal and their distribution width lower than normal. Only the levels of mean cell hemoglobin are within normal ranges. These alterations in red blood cell values and the presence of reticulocytes in the circulation (**Table 2**) suggest that *Gata1*^{low} mice are subjected to stress erythropoiesis for all their life. Further support for this hypothesis is provided by the detailed comparison of the size of the erythroid compartments in the blood, bone marrow and spleen from *Gata1*^{low} mice and their wild-type littermates presented in **Table 4**. This comparison confirmed that in spite of the massive recruitment of the spleen as erythropoietic site, the *Gata1*^{low} mutation specifically reduces the size of the erythroid cells at their early stage of maturation (pro-erythroblasts, basophilic erythroblasts and polychromatic erythroblasts) which undergo high rate of apoptosis even in the spleen, as further indicated by the high numbers of apoptotic cells (by TUNEL staining) present in this organ [7].

There are two types of erythroid stress: acute (such as exposure to rapid changes in altitude or bleeding) and chronic (such as that posed by congenic anemia). Changes in the levels of erythropoietin (EPO) in the circulation occur in response to both stresses, as indicated by a pivotal paper that correlated EPO plasma concentrations and numbers of red blood cells in vivo [68], but effectively normalize the Hct only under acute conditions. In addition to EPO, the response to acute stress requires activation of the glucocorticoid receptor (GR) [69], the soluble form of SCF generated by proteolytic cleavage of the membrane-bound form of this growth factor [70], and the bone morphogenic protein (BMP) pathway [71]. Activation of these pathways cooperates in the generation of stress-specific erythroid progenitors expressing high levels of transforming growth factor β (TGF-β) receptor 3 [72,73]. The clinical patients with gain (Cushing's syndrome) or loss (Addison's disease) of GR function develop erythrocytosis or anemia, respectively [74,75] indicated that GR regulates stress erythropoiesis also in man.

By contrast to the numerous studies on the mechanisms that regulate recovery from acute stress, the signaling activated by chronic erythroid stress is poorly defined. Using as a model of chronic stress thalassemia, the Rivella laboratory demonstrated that chronic stress activates a pathway partially different from that induced by acute stress which relays on JAK2/STAT5 signaling both in mice and men [76].

We took advantage of the fact that *Gata1*^{low} mice are subjected to chronic stress for all their life to test the hypothesis that these mice compensate for inefficient erythropoiesis through a pathway different from that activated during the recover from acute stress. To reach this goal, we first demonstrated that *Gata1*^{low} mice retain the ability to recover from acute stress induced by red blood cell hemolysis following phenylhydrazine treatment or by exogenous administration of recombinant EPO [77](see **Figure 1**). The observation that *Gata1*^{low} mice respond to acute stress, indicate that in these animals the response to chronic stress involves a signaling that does not saturate that activated by the acute response. We then demonstrated that the bone marrow and spleen from *Gata1*^{low} mice do not contain stress-specific progenitor cells but a "unique erythroid" precursor with the morphology of erythroblasts but still capable to mature within 24 hours into megakaryocytes in response to thrombopoietin (TPO) [78,79]. The phenotype of this precursor is similar to that of a bipotent erythroid-megakaryocyte precursors specifically generated in vitro by human CD34^{+} cells in response

to a hyperstimulator of MPL, the receptor for TPO [80], suggesting that chronic stress may activate the TPO/MPL axis. In agreement with this hypothesis, numerous attempts to generate *Gata1*^{low} mice lacking *Mpl* were consistently unsuccessful (ARM, unpublished observations). This hypothesis was tested by determining that *Gata1*^{low} mice contained 2-times more *Tpo* mRNA in liver and TPO in plasma than wild-type littermates **(Table 3)** [81]. Furthermore, *Gata1*^{low} stem/progenitor cells expressed levels of *Mpl* mRNA (5-times greater than normal) and protein (2-times lower than normal) similar to those expressed by cells from TPO-treated wild-type mice and *Gata1*^{low} marrow and spleen contained more JAK2/STAT5 than wild-type tissues, an indication that these organs were reach of TPO-responsive cells.

8. Megakaryocytes from *Gata1*^{low} mice and patients with PMF harbor similar abnormalities

In all backgrounds analyzed so far, *Gata1*^{low} mice remain thrombocytopenic all their life (**Table 2**). In addition, the platelets in the circulation are on average 50% larger (Mean platelet volume 10.70 *vs.* 7.75×10^{-15} L) and are more homogeneous is size (platelet distribution width 15.12 us. 32.04) than normal cells (**Table 2**) [82]. The a-granules of the *Gata1*^{low} platelets are reduced in number and electron density, an indication of poor protein content (**Figure 2**) [83] and contain low levels of both von Willebrand factor and P-selectin [84]. These abnormalities indicate that the *Gata1*^{low} mutation impairs terminal megakaryocyte maturation into platelets.

The hypothesis that reduced levels of GATA1 impair megakaryocyte maturation is provided by the observation that the bone marrow and spleen from Gata1^{low} mice contain twice as many viable megakaryocytes than tissues from normal mice (**Table 4**). The number of megakaryocytes contained in these organs became more than 10-fold greater than normal if para-apoptotic cells (i.e. cells which die by an immune-mediated process which compact the chromatin instead than degrading the DNA as observed in apoptosis) [83]. These megakaryocytes are localized in clusters and have prevalently an immature morphology (**Figure 2**). Few mature Gata1^{low} megakaryocytes are detectable in these tissues but those which are present retain an altered morphology with a poorly developed demarcation membrane system (DMS) and few platelet territories (**Figure 2**) [12,83]. The block in maturation includes failure to properly organize α-granules, since von Willebrand factor is detectable at low levels in mutant cells and P-selectin is found associated with the DMS instead than within α-granules.

An important step in megakaryocyte maturation is assembly of P-selectin and von Willebrand factor in the α-granules where they act as the receptors which trigger the release of their content into the microenvironment when engaged with the P-selectin glycoprotein ligand presented by neutrophils and collagen present in the subendothelial matrix, respectively [85,86]. Electron microscopy studies revealed that TPO stimulates the assembly of von Willebrand factor and P- selectin in separate subsets of α-granules in megakaryocytes from wild-type mice, suggesting that these two receptors may regulate the differential release of pro- and anti-angiogenic platelet factors in response to specific microenvironment

cues, as hypothesized by Italiano et al. [84,87] By contrast, TPO did not alter the localization of the two receptors in *Gata1*^{low} megakaryocytes which remained co-localized in the cytoplasm, suggesting that the mutation deregulate the release of pro- and anti-angiogenic factors for the platelets.

In addition to increased levels of P-selectin expression on the cell surface, other abnormalities displayed by *Gata1*^{low} megakaryocytes include frequent and abnormal emperipoletic interactions with neutrophils and the presence of high levels of TGF-ß in the cytoplasm. Hematology textbooks describe that emperipolesis of neutrophils is observed in 0.8% of the megakaryocytes present in the bone marrow and is used by neutrophils to quickly move from one site to another of the microenvironment using as short cut the DMS of the megakaryocytes. This transient interaction does not alter either the neutrophil or the megakaryocyte involved. In the case of *Gata1*^{low} mice, instead, the process involves up to 30% of the megakaryocytes present both in the bone marrow and in the spleen and is abnormal because the neutrophils remain trapped within the megakaryocytes, fuse their plasma membrane with the DMS releasing the proteolytic content of their α-granules (such as metalloproteinase 9, MPP9) within the megakaryocyte cytoplasm and leading to the death of both cells by papa-apoptosis, an immune-mediated process of cell death which, by contrast with apoptosis, involves high level of chromatin condensation within the nuclei, that became heavily electron dense, and proteolytic leases of the cytoplasm (Centurione et al., 2014). Of note, all of these abnormalities have been observed in megakaryocytes from patients with primary myelofibrosis (PMF), the most severe form of the MPNs, and are considered cellular hallmarks for this disease [88–91].

Surprisingly, in contrast to megakaryocytes purified from the fetal liver [12]. megakaryocytes from adult *Gata1*^{low} mice express apparently normal levels of *GATA1* mRNA (**Table 1**). This result is consistent with the observation that *Gata1*^{low} mice that survive until adulthood activate in the spleen alternative regulatory regions of the gene [67]. However, by immunohistochemistry, megakaryocytes from *Gata1*^{low} mice express barely detectable lezels of GATAI protein [62]. The recognition that hyperactivation of TPO signaling induces a ribosome deficiency that de-regulates GATA1 in megakaryocytes by hampering its translation led us to recognize that *Gata1*^{low} mice also show a ribosomal deficiency and a discordant microarray ribosome signature (reduced ribosomal protein small *(RPS) 24, RPS26* and Shwachman-Bodian-Diamond syndrome protein *(SBDS,* a protein involved in ribosome maturation) expression) which results in megakaryocytes containing a poorly developed endoplasmic reticulum with rare polysomes [81].

Given the already high levels of TPO present in the *Gata1*^{low} mice, it was surprising that pharmacological doses of TPO (25 or 100 μg/kg of body weight) significantly increased platelet counts while reducing hematocrit (Hct) levels (**Figure 1** [92]). In the mutant mice, however, maximal responses were observed three days earlier for the erythroid lineage and 3 or 7 days later for platelets than in wild-type mice, suggesting that the response in mutants involved recruitment of more immature progenitor cell compartments. Changes in platelets counts and hematocrit levels are also induced by treatment with IL-6, the major stimulator of TPO production in vivo [93].

9. *Gata1*^{low} mice develop over time a myelofibrotic phenotype that recapitulates traits of PMF patient

The molecular (reduced GATA1 content) and biological (increased proliferation with reduced maturation) similarities between megakaryocytes from *Gata1*^{low} mice and PMF patients led us to investigate almost 15 years ago whether *Gata1*^{low} mice would develop myelofibrosis with age [62]. These studies established *Gata1*^{low} mice one of the first animal models for the human disease. In contrast to mice overexpressing TPO by a way of retroviral induced expression of the gene in HSCs, TPO^{high} mice (**Table 4**) which experience an accelerated form of the disease and in a timely fashion. For the first 1–8 months, *Gata1*^{low} mice display traits associated with premyelofibrosis such as splenomegaly, increased rates of thrombosis and osteosclerosis. Of note, osteosclerosis in *Gata1*^{low} mice had already been described by Kacena et al. and may be related to the high levels of pro-osteogenic factors expressed by *Gata1*^{low} megakaryocytes [94]. From 8–12-months, they show myelofibrotic traits including fibrosis and neovascularization, and from 12-months until their natural death they express a late- myelofibrotic phenotype which includes increased stem/progenitor cell trafficking and extramedullary hematopoiesis in liver [62]. This progression is similarly observed in hemizygous males and by homozygous/heterozygous females of the CD1 and C57Bl/6 backgrounds [66]. This progression clearly mimics the evolution from a pre-fibrotic (PrePMF) phase to an overt primary myelofibrosis (PMF), which was recognized in this disease by the World Health Organization classification of myeloid neoplasms in 2016 [95].

The discovery that *Gata1*^{low} mice develop myelofibrosis, prompted us to investigate the state of GATA1 in the megakaryocytes from PMF patients. This study identified that megakaryocytes from PMF patients contain levels of GATA1 protein clearly lower than normal [96], indicating that they are indeed hypomorphic for GATA1. *GATA1* mutations, however, were not detected in the numerous patients investigated at that time (Vannucchi and Crispino, unpublished observations), leaving the mechanism which linked the driver mutations in PMF and GATA1 deficiency in megakaryocytes unclear. Other studies revealed that PMF is caused by genetic abnormalities that lead to hyperactive JAK/STAT signaling, such as mutations in *JAK2, MPL,* or calreticulin *(CALR)* [97]. The relationship between PMF and GATA1 deficiency became clear only when it was found that these driver mutations also impair the activity of the ribosome leading to inefficient GATA1 translation in megakaryocytes both in the patients and in animal models [24] (**Figure 3**). Conversely, as mentioned before, more recent observation from our group indicate that the low content of GATAI in the megakaryocytes from adult *Gata1*^{low} mice are likely due to a ribosomal deficiency as a result of activation of JAK/STAT signaling downstream to TPO rather than to the deletion of the HS1 enhancer [24]. Last but not least, mice expressing driver *JAK2* mutations under the control of megakaryocyte-specific enhancer develop myelofibrosis although their HSC are genetically normal, establishing the final causative link in the relationship between driver mutations, hypomorphic GATA1 in megakaryocytes, megakaryocyte abnormalities and development of myelofibrosis.

During the 15 years in which we have used *Gata1*^{low} mice to study the pathogenesis of PMF, we have generated the unifying pathobiological model for the development of the disease

depicted in **Figure 3**. In this model, driver mutations in the patients and chronic stress in *Gata1*^{low} mice activate the TPO/MPL axis that results in RSP14 deficiency, which reduces *GATA1* mRNA translation impairing the maturation of megakaryocytes. Abnormal megakaryocytes in turn express high levels of P-selectin on their surface which triggers neuthrophil emperipolesis leading to increase bioavailability of TGF-β in the microenvironment with consequent fibrocyte activation. Activated fibrocytes are then responsible for fibrosis and hematopoietic failure in the bone marrow and generation of disease-specific HSC niches and amplification of myelofibrotic HSC in the spleen. In fact, as in *Gata1*^{low} mice [67]. the spleen is the major reservoir of myelofibrotic stem cells also in PMF patients [98].

10. Role played by hypomorphic *Gata1*^{low} mice models to identify novel therapeutic targets for PMF

Hypomorphic *Gata1*^{low} mice models, obtained either by directly by deleting the regulatory regions of the gene or indirectly by generating mice carrying driver mutations that induce a ribosomopathy that impairs *Gata1* mRNA translation, are playing an important role in identifying possible molecular targets to cure human PMF. The observation that *Gata1*^{low} mice express an activated TPO/JAK2 pathway led us to investigate the effects of ruxolitinib, a JAK inhibitor approved by the Food and Drug Administration for clinical use in PMF in this model. Similar to those used in *Jak2*^{V617F} animal models [100] greatly reduced spleen size but had modest effects on bone marrow hematopoiesis, suggesting that this drug may be more efficacious when used in combination with other drugs targeting the malignant HSC or their microenvironment.

In the studies published until now, mouse models have provided little evidence for drugs that may specifically target the malignant HSC. The observation that *Gata1*^{low} HSC express reduced levels of p27 (Kipl) a protein downstream to the Rac signaling lead to the discovery that the Rac activator Aplidin, a natural compound isolated from a marine tunica also known as plitidepsin, cured myelofibrosis in this mouse model [101]. Unfortunately, the following clinical trial in PMF patients was ineffective [102].

Gata1^{low} mice and PMF patients express two major micro-environmental abnormalities: They express an abnormal CXCL12/CXCR4 axis that may be responsible for increased stem/progenitor cell trafficking observed in myelofibrosis [103,104] and increased TGF-β bioavailability [88,105–107]. By contrast with the abnormalities in the CXCL12/CXCR4 axis which appear dispensable for the development of the disease [101], TGF-ß has a great pathobiology role in the development of myelofibrosis in animal models [108,109] and two independent studies have demonstrated that TGF-β receptor 1 inhibitors are efficacious in treating myelofibrosis in *Gata1*^{low} [105] and *Jak2*^{V617F} [110] mouse models. Encouraging results were also observed in a limited clinical trial with GC1008 (Fresolimumab, Sanofi Aventis), a human monoclonal antibody that neutralizes the mammalian isoforms of TGF-β [111] that provided evidence of reduced bone marrow fibrosis and sustained improvements in hemoglobin level in at least two of the three patients treated. Unfortunately, the trial was interrupted prematurely due to pharmaceutical company decision to discontinue antibody

production and distribution for clinical investigation. However, new generation TGF-β inhibitors are currently under development and represent interesting therapeutic options, alone or in combination with JAK inhibitors, for PMF [112].

The bone marrow and spleen from *Gata1*^{low} and PMF patients express a similarly abnormal activation of the non-canonical TGF-ß signaling which include high levels of expression of c- JUN [113]. TGF-β activation and altered c-JUN signaling were also recently confirmed in PMF patients and in animal models by the Weissman Laboratory [114]. Since activation of c-JUN may represent one of the signaling which may result in activation of macrophages, which are present in higher frequency in the blood of *Gata1*^{low} mice (**Table 2**), into myelofibrotic-specific niches [115]. drugs targeting c-JUN and macrophages have been proposed as therapeutic strategies for PMF because they may be more specific and have less toxic effects than TGF-β inhibitor [114].

In addition to TGF-β, most of the other pathobiology elements identified by mouse models in the disease discussed in **Figure 3** have been investigated as a potential therapeutic target for myelofibrosis and some of them are presently in clinical trials in PMF. Below we discuss some of the results obtained up to now.

10.1. P-selectin.

High levels of P-selectin expression have been demonstrated to increase the risk of thrombosis and to contribute to the development of myelofibrosis in the *Gata1*^{low} mouse model. In fact, deletion of the P-selectin gene reduced the risk for thrombosis [116] and prevented development of myelofibrosis [117] in this model. These results are interesting because suggest that the P-selectin inhibitor developed by Novartis which effectively prevents pain crisis in Sickle Cell Disease [118] may also an interesting candidate for treatment of PMF patients.

10.2. Neutrophils.

Activation of non-canonical TGF-β signaling similar to that obser *Gata1*^{low} mice and PMF patients is often observed in animal models of fibrosis related to autoimmunity [119,120]. Conversely patients with autoimmune diseases have increased risk to develop PMF [121], and the plasma from PMF patients contains levels of mitochondrial DNA and antimitochondrial antibodies greater than normal [113]. These results suggest that an autoimmune process may contribute to the development of marrow fibrosis in PMF. However, this hypothesis is counterintuitive in view of the fact that the dendritic cells in the blood from *Gata1*^{low} mice are unable to present antigens and, therefore to activate T cells [10], while blood from PMF patients contain levels 2-fold lower than normal of regulatory T cells with the phenotype $CD41^{+}CD25^{bright}CD127^{low}FOXP3^{+}$ which are needed to initiate autoimmune reactions [122]. Therefore, if autoimmunity is involved in PMF, then T cells must be activated by an alternative pathway.

Not surprisingly given the inhibitory effects exerted by GATA1 on the development of cells of the myelomonocytic lineages, blood from *Gata1*^{low} mice contain levels greater than normal, not only of monocytes, but also of neutrophils while the number of T cells in these

mice is within the normal range (**Table 2**). We hypothesized that in myelofibrosis these abnormally high numbers of neutrophils during their interaction with para-apoptotic megakaryocytes would interact with mitochondrial DNA and generate neutrophil presenting extracellular trap (NET) of autologous DNA which would be responsible for antigen presentation and activation of T cells triggering the auto-immune-reactions. Indeed evidence for neutrophil extracellular trap formation in patients with MPN has been recently identified by Oyarzun et al. [123]

11. Conclusions

GATA1 is an essential regulator of hematopoiesis that participates in development of a number of blood lineages. Alterations in GATA1 in the erythroid lineage typically result in a reduction in the number of erythroid progenitors and anemia. By contrast, although loss of GATA1 in megakaryocytes results in thrombocytopenia, there is a marked increase in the abundance of immature megakaryocytes. This latter feature is prominently displayed in patients with myelofibrosis. Given the strikingly similar disease course in *Gata1*^{low} mice and MF, the hypothesis that a reduction in GATA1 may underlie myelofibrosis was reasonable. Indeed, a number of studies have now shown that GATA1 protein, but not mRNA, is reduced in the megakaryocytes of patients [24,96]. More recent work has established that there exists a link between GATA1 downregulation and the ribosome [81]. These insights may lead the way to development of new strategies to increase GATA1 expression as a means to correct the megakaryocyte defect. Such methods may, in combination with existing and investigational agents, improve outcomes for patients with MF.

12. Expert Commentary

The discovery of *JAK2* mutations led many to suspect that JAK kinase inhibitors would provide substantial, durable anti-tumor activity in the MPNs, similar to the success with imatinib in CML. However, several limitations have been observed. First, to date only one inhibitor, ruxolitinib, has been approved for PMF. Second, ruxolitinib does not consistently reduce the mutant allele burden, despite clear genetic data indicating that JAK2 is required for the mutant hematopoietic cells. Third, there is a dose limiting toxicity owning to an on-target effect on hematopoietic cells, which require JAK2 for red blood cell and platelet production. Fourth, the benefit for the majority of patients wanes in the second and third year. Taken together, new therapies, such as mutant specific inhibitors, immunotherapy or novel combinatorial treatments are needed.

There are several challenges that need to be addressed in order to improve the outcom patients. One major challenge to the field is understanding how one mutation, such as JAK2V617F, causes three different, albeit closely related, diseases. It is possible that the mutation occurs in different types of progenitors in different patients. An example of that could be the acquisition of a *JAK2, MPL* or *CALR* mutation selectively in megakaryocyte-biased hematopoietic stem cells. This event might lead to PMF or essential thrombocythemia (ET), but not polycythemia vera (PV). Alternatively, the disease difference might be due to hitherto uncharacterized secondary genetic events or epigenetic differences among individuals. Another significant challenge in the management of the MPNs is the sudden

progression to a acute myeloid leukemia, which occurs in as many as 20% of MF patients. Unfortunately, there are few ways to treat these patients and even fewer ways to predict who will progress and when. The focus of preventing this transition should be the aggressive pursuit of targeting the *JAK2* mutant malignant stem cell population, which is currently not accomplished by current therapies. On the other hand, one must keep in mind that many cases of post-MPN AML in patients with *JAK2* mutations present without a *JAK2* mutation. These findings suggest that MPN patients may present with a pro-leukemic milieu or that the AML arises from a pre-JAK2V617F malignant clone.

With regard to the major focus of this review, the role of megakaryocytes and GATA1 in PMF, we anticipate a number of advances in the coming years. First, novel agents that act at least in part by targeting this lineage, such as the Aurora A kinase inhibitor alisertib, are being tested in Phase 1 studies of PMF patients. Additional studies to target TGF-β, which is secreted by PMF megakaryocytes at relatively high levels, are well underway. The results of these studies may provide significant insights into the role of megakaryocytes in this malignancy.

Finally, DBA is a rare inherited disorder that is primarily associated with ribosomal mutations, but in some cases harbors a *GATA1* mutation that, like the ones in AMKL, result in unique expression of the GATA1 s isoform. Notable studies by Sankaran and colleagues have shown that a key effect of ribosomal deficiency in DBA is the impaired translation of GATA1, providing a link between these two classes of mutations. It is interesting to note that the GATA1 deficiency in MF is also associated with ribosomal defects. Further studies to characterize the link between JAK/STAT activation, ribosomes and GATA1 are needed to better define the mechanism of impaired megakaryopoiesis in the MPNs.

13. Five-year view

Over the next five years, we expect to see the advent of new therapies for the MPNs. The most likely scenario is success in one of the relatively large number of studies to combine novel agents with ruxolitinib. We may also finally witness the approval of a second line of JAK inhibitors, which will hopefully provide benefit to patients who progress despite ruxolitinib treatment. It is safe to say that there will be many new biological insights into the disease in the near future. This may include insights into what drives progression of ET and PV into PMF, and what promotes the evolution of the MPNs to AML. Also, we anticipate a better understanding of the cell lineages that promote fibrosis, which should provide ideas on how to suppress or even reverse the process. The number of researchers in the field is growing, so the pace of biological discoveries should accelerate in parallel.

14. Key Issues

Acknowledgements

We wish to thank Prof. Alessandro Vannucchi, for assisting in the analyses of the treatment with interleukin-6 and low dose TPO. Dr. Vannucchi provided written consent for the data to be described in this manuscript.

Funding

This work was supported by grants from the National Cancer Institute (P01-CA108671) and Associazione Italiana Ricerca Cancro (AIRC 17608) to ARM and DK101329 to JDC.

References

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Crispino JD, Horwitz MS. GATA factor mutations in hematologic disease. Blood.2017;129:2103–10 [PubMed: 28179280]
2. Kerenyi MA, Orkin SH. Networking erythropoiesis. J Exp Med. 2010;207:2537–41 [PubMed: 21098097]
3. Kadri Z, Shimizu R, Ohneda O et al. Direct binding of pRb/E2F-2 to GATA-1 regulates maturation and terminal cell division during erythropoiesis. PLoS Biol. 2009;7:e1000123 [PubMed: 19513100]
4. Migliaccio AR, Migliaccio G, Ashihara E et al. Erythroid-specific activation of the distal (testis) promoter of GATA1 during differentiation of purified normal murine hematopoietic stem cells. Acta Haematol. 1996;95:229–35 [PubMed: 8677748]
*5. Attema JL, Papathanasiou P, Forsberg EC et al. Epigenetic characterization of hematopoietic stem cell differentiation using miniChIP and bisulfite sequencing analysis. Proc Natl Acad Sci U S A. 2007;104:12371–6

This paper confirmed tgat Gata1 is expressed in murine hematopoietic stem cells

[PubMed: 17640913]
6. Yu C, Cantor AB, Yang H et al. Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. J Exp Med. 2002;195:1387–95 [PubMed: 12045237]
7. Migliaccio AR, Rana RA, Sanchez M et al. GATA-1 as a regulator of mast cell differentiation revealed by the phenotype of the GATA-1low mouse mutant. J Exp Med. 2003;197:281–96 [PubMed: 12566412]
8. Ohneda K, Moriguchi T, Ohmori S et al. Transcription factor GATA1 is dispensable for mast cell differentiation in adult mice. Mol Cell Biol. 2014;34:1812–26 [PubMed: 24615013]
9. Scheenstra MR, De Cuyper IM, Branco-Madeira F et al. GATA1-Deficient Dendritic Cells Display Impaired CCL21-Dependent Migration toward Lymph Nodes Due to Reduced Levels of Polysialic Acid. J Immunol. 2016;197:4312–24 [PubMed: 27815426]
10. Kozma GT, Martelli F, Verrucci M et al. Dynamic regulation of Gata1 expression during the maturation of conventional dendritic cells. Exp Hematol. 2010;38:489–503.e1 [PubMed: 20303380]
11. Lee WY, Weinberg OK, Pinkus GS. GATA1 Is a Sensitive and Specific Nuclear Marker for Erythroid and Megakaryocytic Lineages. Am J Clin Pathol. 2017;147:420–6 [PubMed: 28340113]
*12. Shivdasani RA, Fujiwara Y, McDevitt MA et al. A lineage-selective knockout establishes the critical role of transcription factor GATA-1 in megakaryocyte growth and platelet development. EMBO J. 1997;16:3965–73

This paper characterized the alterations induced by reduced GATA1 expression in megakaryocyte development

[PubMed: 9233806]
13. Simon MC, Pevny L, Wiles MV et al. Rescue of erythroid development in gene targeted GATA-1- mouse embryonic stem cells. Nat Genet. 1992;1:92–8 [PubMed: 1302015]
14. Fujiwara Y, Browne CP, Cunniff K et al. Arrested development of embryonic red cell precursors in mouse embryos lacking transcription factor GATA-1. Proc Natl Acad Sci U S A. 1996;93:12355–8 [PubMed: 8901585]

15. Yamaguchi Y, Zon LI, Ackerman SJ et al. Forced GATA-1 Expression in the Murine Myeloid Cell Line Ml: Induction of c-Mpl Expression and Megakaryocytic/Erythroid Differentiation. Blood. 1998;91:450–7 [PubMed: 9427697]

16. Layon ME, Ackley CJ, West RJ et al. Expression of GATA-1 in a Non-Hematopoietic Cell Line Induces β-Globin Locus Control Region Chromatin Structure Remodeling and an Erythroid Pattern of Gene Expression. J Mol Biol. 2007;366:737–44 [PubMed: 17196618]

17. Visvader JE, Elefanty AG, Strasser A et al. GATA-1 but not SCL induces megakaryocytic differentiation in an early myeloid line. EMBO J. 1992; 11:4557–64 [PubMed: 1385117]

18. Crispino JD, Weiss MJ. Erythro-megakaryocytic transcription factors associated with hereditary anemia. Blood. 2014;123:3080–8 [PubMed: 24652993]

19. Campbell AE, Wilkinson-White L, Mackay JP et al. Analysis of disease-causing GATA1 mutations in murine gene complementation systems. Blood. 2013;121:5218–27 [PubMed: 23704091]

20. Calligaris R, Bottardi S, Cogoi S et al. Alternative translation initiation site usage results in two functionally distinct forms of the GATA-1 transcription factor. Proc Natl Acad Sci USA. 1995;92:11598–602 [PubMed: 8524811]

21. Chlon TM, McNulty M, Goldenson B et al. Global transcriptome and chromatin occupancy analysis reveal the short isoform of GATAI is deficient for erythroid specification and gene expression. Haematologica. 2015;100:575–84 [PubMed: 25682601]

*22. Byrska-Bishop M, VanDorn D, Campbell AE et al. Pluripotent stem cells reveal erythroid-specific activities of the GATAI N-terminus. J Clin Invest. 2015;125:993–1005

This paper characterized the distinctive functions of the two GATA1 isoforms

[PubMed: 25621499]

**23. Ludwig LS, Gazda HT, Eng JC et al. Altered translation of GATAI in Diamond- Blackfan anemia. Nat Med. 2014;20:748–53

This paper was the first to identify the mechanisms which may induce hypomorphic GATAI levels in human diseases

[PubMed: 24952648]

**24. Gilles L, Arslan AD, Marinaccio C et al. Downregulation of GATAI drives impaired hematopoiesis in primary myelofibrosis. J Clin Invest. 2017;127:1316–20

This paper identified hypomorphic GATAI levels in animal models and in patients with primary myelofibrosis

[PubMed: 28240607]

25. O'Brien KA, Farrar JE, Vlachos A et al. Molecular convergence in ex vivo models of Diamond-Blackfan anemia. Blood. 2017;129:3111–20 [PubMed: 28377399]

26. Cantor AB, Orkin SH. Transcriptional regulation of erythropoiesis: an affair involving multiple partners. Oncogene. 2002;21:3368–76 [PubMed: 12032775]

27. Shimizu R, Yamamoto M. GATA-related hematologic disorders. Exp Hematol. 2016;44:696–705 [PubMed: 27235756]

*28. Bresnick EH, Lee HY, Fujiwara T et al. GATA switches as developmental drivers. J Biol Chem. 2010;285:31087–93

This paper identified that progression of hematopoietic differentation requires a GATA2 to GATAI switch

[PubMed: 20670937]

29. Grass JA, Boyer ME, Pal S et al. GATA-1-dependent transcriptional repression of GATA-2 via disruption of positive autoregulation and domain-wide chromatin remodeling. Proc Natl Acad Sci USA. 2003;100:8811–6 [PubMed: 12857954]

30. Ohyashiki K, Ohyashiki JH, Shimamoto T et al. Pattern of expression and their clinical implications of the GATA family, stem cell leukemia gene, and EVI1 in leukemia and myelodysplastic syndromes. Leuk Lymphoma. 1996;23:431–6 [PubMed: 9031072]

31. Li Z, Godinho FJ, Klusmann JH et al. Developmental stage-selective effect of somatically mutated leukemogenic transcription factor GATA1. Nat Genet. 2005;37:613–9 [PubMed: 15895080]

32. Birger Y, Goldberg L, Chlon TM et al. Perturbation of fetal hematopoiesis in a mouse model of Down syndrome's transient myeloproliferative disorder. Blood. 2013;122:988–98 [PubMed: 23719302]

33. Kadauke S, Blobel GA. "Remembering" tissue-specific transcription patterns through mitosis. Cell Cycle. 2012;11:3911–2 [PubMed: 23032269]

*34. Kadauke S, Udugama MI, Pawlicki JM et al. Tissue-Specific Mitotic Bookmarking by Hematopoietic Transcription Factor GATA1. Cell. 2012;150:725–37

This paper identified GATA1 as the lineage bookmarker after mitosis

[PubMed: 22901805]

35. Lejon S, Thong SY, Murthy A et al. Insights into Association of the NuRD Complex with FOG-1 from the Crystal Structure of an RbAp48-FOG-1 Complex. J Biol Chem. 2011;286:1196–203 [PubMed: 21047798]

36. Ross J, Mavoungou L, Bresnick EH et al. GATA-1 Utilizes Ikaros and Polycomb Repressive Complex 2 To Suppress Hes1 and To Promote Erythropoiesis. Mol Cell Biol. 2012;32:3624–38 [PubMed: 22778136]

37. Xu J, Shao Z, Li D et al. Developmental control of polycomb subunit composition by GATA factors mediates a switch to non-canonical functions. Mol Cell. 2015;57:304–16 [PubMed: 25578878]

38. Cheng Y, Wu WS, Kumar SA et al. Erythroid GATA1 function revealed by genomewide analysis of transcription factor occupancy, histone modifications, and mRNA expression. Genome Res. 2009;19:2172–84 [PubMed: 19887574]

39. Papadopoulos GL, Karkoulia E, Tsamardinos I et al. GATA-1 genome-wide occupancy associates with distinct epigenetic profiles in mouse fetal liver erythropoiesis. Nucleic Acids Res. 2013;41:4938–48 [PubMed: 23519611]

40. Hong W, Nakazawa M, Chen YY et al. FOG-1 recruits the NuRD repressor complex to mediate transcriptional repression by GATA-1. EMBO J. 2005;24:2367–78 [PubMed: 15920470]

41. Yang T, Jian W, Luo Y et al. Acetylation of histone deacetylase 1 regulates NuRD corepressor complex activity. J Biol Chem. 2012;287:40279–91 [PubMed: 23014989]

42. Margueron R, Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature. 2011;469:343–9 [PubMed: 21248841]

43. Yu M, Riva L, Xie H et al. Insights into GATA-1 Mediated Gene Activation versus Repression via Genome-wide Chromatin Occupancy Analysis. Mol Cell. 2009;36:682–95 [PubMed: 19941827]

44. Yu W, Zhang F, Wang S et al. Depletion of polycomb repressive complex 2 core component EED impairs fetal hematopoiesis. Cell Death Dis. 2017;8:e2744 [PubMed: 28406475]

45. Varricchio L, Dell'Aversana C, Nebbioso A et al. Identification of NuRSERY, a new functional HDAC complex composed by HDAC5, GATA1, EKLF and pERK present in human erythroid cells. Int J Biochem Cell Biol. 2014;50:112–22 [PubMed: 24594363]

46. Han X, Zhang J, Peng Y et al. Unexpected role for p19INK4d in posttranscriptional regulation of GATA1 and modulation of human terminal erythropoiesis. Blood. 2017;129:226–37 [PubMed: 27879259]

47. Mundschau G, Crispino J. GATA1s goes germline. Nat Genet. 2006;38:741–2 [PubMed: 16804537]

48. Sankaran VG, Ghazvinian R, Do R et al. Exome sequencing identifies GATA1 mutations resulting in Diamond-Blackfan anemia. J Clin Invest. 2012;122:2439–43 [PubMed: 22706301]

49. Wechsler J, Greene M, McDevitt MA et al. Acquired mutations in GATA1 in the megakaryoblastic leukemia of Down syndrome. Nat Genet. 2002;32:148–52 [PubMed: 12172547]

50. Mundschau G, Gurbuxani S, Gamis AS et al. Mutagenesis of GATAI is an initiating event in Down syndrome leukemogenesis. Blood. 2003;101:4298–300 [PubMed: 12560215]

51. Muntean AG, Crispino JD. Differential requirements for the activation domain and FOG-interaction surface of GATA-1 in megakaryocyte gene expression and development. Blood. 2005;106:1223–31 [PubMed: 15860665]

52. Kuhl C, Atzberger A, Iborra F et al. GATA1-mediated megakaryocyte differentiation and growth control can be uncoupled and mapped to different domains in GATA1. Mol Cell Biol. 2005;25:8592–606 [PubMed: 16166640]

53. Hollanda LM, Lima CS, Cunha AF et al. An inherited mutation leading to production of only the short isoform of GATA-1 is associated with impaired erythropoiesis. Nat Genet. 2006;38:807–12 [PubMed: 16783379]

54. Ferreira R, Wai A, Shimizu R et al. Dynamic regulation of Gata factor levels is more important than their identity. Blood. 2007;109:5481–90 [PubMed: 17327407]

55. Nicolis S, Bertini C, Ronchi A et al. An erythroid specific enhancer upstream to the gene encoding the cell-type specific transcription factor GATA-1. Nucleic Acids Res. 1991;19:5285–91 [PubMed: 1656391]

56. Ferreira R, Ohneda K, Yamamoto M et al. GATA1 function, a paradigm for transcription factors in hematopoiesis. Mol Cell Biol. 2005;25:1215–27 [PubMed: 15684376]

57. Kobayashi M, Yamamoto M. Regulation of GATA1 gene expression. J Biochem. 2007;142:1–10 [PubMed: 17567656]

58. Guyot B, Murai K, Fujiwara Y et al. Characterization of a megakaryocyte-specific enhancer of the key hemopoietic transcription factor GATA1. J Biol Chem. 2006;281:13733–42 [PubMed: 16551635]

59. Takahashi S, Onodera K, Motohashi H et al. Arrest in primitive erythroid cell development caused by promoter-specific disruption of the GATA-1 gene. J Biol Chem. 1997;272:12611–5 [PubMed: 9139715]

*60. McDevitt MA, Shivdasani RA, Fujiwara Y et al. A “knockdown” mutation created by cis-element gene targeting reveals the dependence of erythroid cell maturation on the level of transcription factor GATA-1. Proc Natl Acad Sci U S A. 1997;94:6781–5

This was the first paper that used hypomorphic mutations to characterize gene functions

[PubMed: 9192642]

61. Shimizu R, Kuroha T, Ohneda O et al. Leukemogenesis caused by incapacitated GATA-1 function. Mol Cell Biol. 2004;24:10814–25 [PubMed: 15572684]

62. Vannucchi AM, Bianchi L, Cellai C et al. Development of myelofibrosis in mice genetically impaired for GATA-1 expression (GATA-1(low) mice). Blood. 2002;100:1123–32 [PubMed: 12149188]

63. Ghinassi B, Sanchez M, Martelli F et al. The hypomorphic Gata1low mutation alters the proliferation/differentiation potential of the common megakaryocytic-erythroid progenitor. Blood. 2007;109:1460–71 [PubMed: 17038527]

64. Noh JY, Gandre-Babbe S, Wang Y et al. Inducible Gata1 suppression expands megakaryocyte-erythroid progenitors from embryonic stem cells. J Clin Invest. 2015;125:2369–74 [PubMed: 25961454]

65. Eliades A, Papadantonakis N, Bhupatiraju A et al. Control of megakaryocyte expansion and bone marrow fibrosis by lysyl oxidase. J Biol Chem. 2011;286:27630–8 [PubMed: 21665949]

66. Martelli F, Ghinassi B, Panetta B et al. Variegation of the phenotype induced by the Gata1low mutation in mice of different genetic backgrounds. Blood. 2005;106:4102–13 [PubMed: 16109774]

67. Migliaccio AR, Martelli F, Verrucci M et al. Gata1 expression driven by the alternative HS2 enhancer in the spleen rescues the hematopoietic failure induced by the hypomorphic Gata1low mutation. Blood. 2009;114:2107–20 [PubMed: 19571316]

68. Erslev AJ, Wilson J, Caro J. Erythropoietin titers in anemic, nonuremic patients. J Lab Clin Med. 1987;109:429–33 [PubMed: 3102659]

69. Bauer A, Tronche F, Wessely O et al. The glucocorticoid receptor is required for stress erythropoiesis. Genes Dev. 1999;13:2996–3002 [PubMed: 10580006]

70. Tajima Y, Moore MA, Soares V et al. Consequences of exclusive expression in viv Kit-ligand lacking the major proteolytic cleavage site. Proc Natl Acad Sci U S 1998;95:11903–8

71. Paulson RF, Shi L, Wu DC. Stress erythropoiesis: new signals and new st cells. Curr Opin Hematol. 2011;18:139–45 [PubMed: 21372709]

72. Gao X, Lee HY, da Rocha EL et al. TGF-beta inhibitors stimulate re production by enhancing self-renewal of BFU-E erythroid progenitor 2016;128:2637–41

73. Perry JM, Harandi OF, Paulson RF. BMP4, SCF, and hypoxia coo expansion of murine stress erythroid progenitors. Blood. 2007;

74. Gursoy A, Dogruk Unal A, Ayturk S et al. Polycythemia as the first manifestation of Cushing's disease. J Endocrinol Invest. 2006;29:742–4 [PubMed: 17033265]

75. Ellis H Thomas Addison: Addisonian (pernicious) anaemia, Addison's disease of the suprarenal gland. J Perioper Pract. 2013;23:31–2 [PubMed: 23413534]

76. Ramos P, Casu C, Gardenghi S et al. Macrophages support pathological erythropoiesis in polycythemia vera and beta-thalassemia. Nat Med. 2013;19:437–45 [PubMed: 23502961]

77. Vannucchi AM, Bianchi L, Cellai C et al. Accentuated response to phenylhydrazine and erythropoietin in mice genetically impaired for their GATA-1 expression (GATA-1(low) mice). Blood. 2001;97:3040–50 [PubMed: 11342429]

78. Vannucchi AM, Paoletti F, Linari S et al. Identification and characterization of a bipotent (erythroid and megakaryocytic) cell precursor from the spleen of phenylhydrazine-treated mice. Blood. 2000;95:2559–68 [PubMed: 10753835]

79. Sanchez M, Weissman IL, Pallavicini M et al. Differential amplification of murine bipotent megakaryocytic/erythroid progenitor and precursor cells during recovery from acute and chronic erythroid stress. Stem Cells. 2006;24:337–48 [PubMed: 16144876]

80. Belay E, Miller CP, Kortum AN et al. A hyperactive Mpl-based cell growth switch drives macrophage-associated erythropoiesis through an erythroid-megakaryocytic precursor. Blood. 2015;125:1025–33 [PubMed: 25343958]

81. Zingariello M, Sancillo L, Martelli F et al. The thrombopoietin/MPL axis is activated in the Gata1low mouse model of myelofibrosis and is associated with a defective RPS14 signature. Blood Cancer J. 2017;7:e572 [PubMed: 28622305]

82. Vyas P, Ault K, Jackson CW et al. Consequences of GATA-1 deficiency in megakaryocytes and platelets. Blood. 1999;93:2867–75 [PubMed: 10216081]

83. Centurione L, Di Baldassarre A, Zingariello M et al. Increased and pathologic emperipolesis of neutrophils within megakaryocytes associated with marrow fibrosis in GATA-1(low) mice. Blood. 2004;104:3573–80 [PubMed: 15292068]

84. Zingariello M, Fabucci ME, Bosco D et al. Differential localization of P-selectin and von Willebrand factor during megakaryocyte maturation. Biotech Histochem. 2010;85:157–70 [PubMed: 20426698]

85. Hop C, Guilliatt A, Daly M et al. Assembly of multimeric von Willebrand factor directs sorting of P-selectin. Arterioscler Thromb Vasc Biol. 2000;20:1763–8 [PubMed: 10894814]

86. Denis CV, Andre P, Saffaripour S et al. Defect in regulated secretion of P-selectin affects leukocyte recruitment in von Willebrand factor-deficient mice. Proc Natl Acad Sci U S A. 2001;98:4072–7 [PubMed: 11274431]

87. Italiano JE, Jr., Richardson JL, Patel-Hett S et al. Angiogenesis is regulated by a novel mechanism: pro- and antiangiogenic proteins are organized into separate platelet alpha granules and differentially released. Blood. 2008;111:1227–33 [PubMed: 17962514]

**88. Schmitt A, Jouault H, Guichard J et al. Pathologic interaction between megakaryocytes and polymorphonuclear leukocytes in myelofibrosis. Blood. 2000;96:1342–7

This paper identified for the time time an abnormal pathological interaction between megakarocytes and neutrophil responsible to increase TGF-β bioavailability in primary myelofibrosis

[PubMed: 10942376]

89. Thiele J, Lorenzen J, Manich B et al. Apoptosis (programmed cell death) in idiopathic (primary) osteo-/myelofibrosis: naked nuclei in megakaryopoiesis reveal features of para- apoptosis. Acta Haematol. 1997;97:137–43 [PubMed: 9066708]

90. Schmitt A, Drouin A, Masse JM et al. Polymorphonuclear neutrophil and megakaryocyte mutual involvement in myelofibrosis pathogenesis. Leuk Lymphoma. 2002;43:719–24 [PubMed: 12153156]

91. Alvarez-Larran A, Arellano-Rodrigo E, Reverter JC et al. Increased platelet, leukocyte, and coagulation activation in primary myelofibrosis. Ann Hematol. 2008;87:269–76 [PubMed: 17899078]
92. Vannucchi AM, Bianchi L, Paoletti F et al. A pathobiologic pathway linking thrombopoietin, GATA-1, and TGF-beta1 in the development of myelofibrosis. Blood. 2005;105:3493–501 [PubMed: 15665119]
93. Kaser A, Brandacher G, Steurer W et al. Interleukin-6 stimulates thrombopoiesis through thrombopoietin: role in inflammatory thrombocytosis. Blood. 2001;98:2720–5 [PubMed: 11675343]
94. Kacena MA, Shivdasani RA, Wilson K et al. Megakaryocyte-osteoblast interaction revealed in mice deficient in transcription factors GATA-1 and NF-E2. J Bone Miner Res. 2004;19:652–60 [PubMed: 15005853]
95. Arber DA, Orazi A, Hasserjian R et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127:2391–405 [PubMed: 27069254]
96. Vannucchi AM, Pancrazzi A, Guglielmelli P et al. Abnormalities of GATA-1 in Megakaryocytes from Patients with Idiopathic Myelofibrosis. Am J Pathol. 2005;167:849–58 [PubMed: 16127162]
97. Grinfeld J, Nangalia J, Green AR. Molecular determinants of pathogenesis and clinical phenotype in myeloproliferative neoplasms. Haematologica. 2017;102:7–17 [PubMed: 27909216]
98. Wang X, Prakash S, Lu M et al. Spleens of myelofibrosis patients contain malignant hematopoietic stem cells. J Clin Invest. 2012;122:3888–99 [PubMed: 23023702]
99. Vannucchi AM, Kantarjian HM, Kiladjian J-J et al. A pooled analysis of overall survival in COMFORT-I and COMFORT-II, 2 randomized phase III trials of ruxolitinib for the treatment of myelofibrosis. Haematologica. 2015;100:1139–45 [PubMed: 26069290]
100. Quintás-Cardama A, Vaddi K, Liu P et al. Preclinical characterization of the selective JAK1/2 inhibitor INCB018424: therapeutic implications for the treatment of myeloproliferative neoplasms. Blood. 2010;115:3109–17 [PubMed: 20130243]
101. Verrucci M, Pancrazzi A, Aracil M et al. CXCR4-Independent Rescue of the Myeloproliferative Defect of the Gata1(low) Myelofibrosis Mouse Model by Aplidin(®). J Cell Physiol. 2010;225:490–9 [PubMed: 20458749]
102. Pardanani A, Tefferi A, Guglielmelli P et al. Evaluation of plitidepsin in patients with primary myelofibrosis and post polycythemia vera/essential thrombocythemia myelofibrosis: results of preclinical studies and a phase II clinical trial. Blood Cancer J. 2015;5:e286 [PubMed: 25768401]
103. Migliaccio AR, Martelli F, Verrucci M et al. Altered SDF-1/CXCR4 axis in patients with primary myelofibrosis and in the Gata1 low mouse model of the disease. Exp Hematol. 2008;36:158–71 [PubMed: 18206727]
104. Abdelouahab H, Zhang Y, Wittner M et al. CXCL12/CXCR4 pathway is activated by oncogenic JAK2 in a PI3K-dependent manner. Oncotarget. 2017;8:54082–95 [PubMed: 28903325]
105. Zingariello M, Martelli F, Ciaffoni F et al. Characterization of the TGF-beta1 signaling abnormalities in the Gata1low mouse model of myelofibrosis. Blood. 2013;121:3345–63 [PubMed: 23462118]
106. Ciurea SO, Merchant D, Mahmud N et al. Pivotal contributions of megakaryocytes to the biology of idiopathic myelofibrosis. Blood. 2007;110:986–93 [PubMed: 17473062]
107. Chagraoui H, Komura E, Tulliez M et al. Prominent role of TGF-beta 1 in thrombopoietin-induced myelofibrosis in mice. Blood. 2002;100:3495–503 [PubMed: 12393681]
108. Vannucchi AM, Villeval J-L, Wagner-Ballon O et al. Animal Models of Myelofibrosis In: Sourcebook of Models for Biomedical Research. Conn PM (Ed. (Humana Press, Totowa, NJ). 2008;713–23
109. Vainchenker W, Constantinescu SN, Plo I. Recent advances in understanding myelofibrosis and essential thrombocythemia. F1000Research. 2016;5:F1000 Faculty Rev-700
110. Yue L, Bartenstein M, Zhao W et al. Efficacy of ALK5 inhibition in myelofibrosis. JCI Insight. 2017;2:e90932 [PubMed: 28405618]

111. Mascarenhas J, Li T, Sandy L et al. Anti-transforming growth factor-beta therapy in patients with myelofibrosis. Leuk Lymphoma. 2014;55:450–2 [PubMed: 23682558]

112. Ceglia I, Dueck AC, Masiello F et al. Preclinical rationale for TGF-beta inhibition as a therapeutic target for the treatment of myelofibrosis. Exp Hematol. 2016;44:1138–55.e4 [PubMed: 27592389]

113. Ciaffoni F, Cassella E, Varricchio L et al. Activation of non-canonical TGF-beta1 signaling indicates an autoimmune mechanism for bone marrow fibrosis in primary myelofibrosis. Blood Cells Mol Dis. 2015;54:234–41 [PubMed: 25703685]

**114. Wernig G, Chen SY, Cui L et al. Unifying mechanism for different fibrotic diseases. Proc Natl Acad Sci U S A. 2017;114:4757–62

This paper identified c-Jun as a common abnormality across fibrosis in multiple organs

[PubMed: 28424250]

115. Verstovsek S, Manshouri T, Pilling D et al. Role of neoplastic monocyte-derived fibrocytes in primary myelofibrosis. J Exp Med. 2016;213:1723–40 [PubMed: 27481130]

116. Zetterberg E, Verrucci M, Martelli F et al. Abnormal P-selectin localization during megakaryocyte development determines thrombosis in the gata1low model of myelofibrosis. Platelets. 2014;25:539–47 [PubMed: 24176039]

117. Spangrude GJ, Lewandowski D, Martelli F et al. P-Selectin Sustains Extramedullary Hematopoiesis in the Gata1 low Model of Myelofibrosis. Stem Cells. 2016;34:67–82 [PubMed: 26439305]

118. Ataga KI, Kutlar A, Kanter J et al. Crizanlizumab for the Prevention of Pain Crises in Sickle Cell Disease. N Engl J Med. 2017;376:429–39 [PubMed: 27959701]

119. Gerber EE, Gallo EM, Fontana SC et al. Integrin-modulating therapy prevents fibrosis and autoimmunity in mouse models of scleroderma. Nature. 2013;503:126–30 [PubMed: 24107997]

120. Akhmetshina A, Palumbo K, Dees C et al. Activation of canonical Wnt signalling is required for TGF-beta-mediated fibrosis. Nat Commun. 2012;3:735 [PubMed: 22415826]

121. Barosi G An immune dysregulation in MPN. Curr Hematol Malig Rep. 2014;9:331–9 [PubMed: 25139710]

122. Massa M, Campanelli R, Fois G et al. Reduced frequency of circulating CD4+CD25brightCD127lowFOXP3+ regulatory T cells in primary myelofibrosis. Blood. 2016;128:1660–2 [PubMed: 27531678]

123. Marin Oyarzùn CP, Carestia A, Lev PR et al. Neutrophil extracellular trap formation and circulating nucleosomes in patients with chronic myeloproliferative neoplasms. Sci Rep. 2016;6:38738 [PubMed: 27958278]

124. Bersenev A, Wu C, Balcerek J et al. Lnk constrains myeloproliferative diseases in mice. J Clin Invest. 2010;120:2058–69 [PubMed: 20458146]

125. Yang Y, Akada H, Nath D et al. Loss of Ezh2 cooperates with Jak2V617F in the development of myelofibrosis in a mouse model of myeloproliferative neoplasm. Blood. 2016;127:3410–23 [PubMed: 27081096]

126. Shimizu T, Kubovcakova L, Nienhold R et al. Loss of Ezh2 synergizes with JAK2- V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. J Exp Med. 2016;213:1479–96 [PubMed: 27401344]

127. Marty C, Harini N, Pecquet C et al. Calr Mutants Retroviral Mouse Models Lead to a Myeloproliferative Neoplasm Mimicking an Essential Thrombocythemia Progressing to a Myelofibrosis. Blood. 2014;124:157– [PubMed: 25013158]

128. Akada H, Yan D, Zou H et al. Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. Blood. 2010;115:3589–97 [PubMed: 20197548]

129. Li J, Spensberger D, Ahn JS et al. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. Blood. 2010;116:1528–38 [PubMed: 20489053]

130. Bumm TG, Elsea C, Corbin AS et al. Characterization of murine JAK2V617F-positive myeloproliferative disease. Cancer Res. 2006;66:11156–65 [PubMed: 17145859]

131. Lacout C, Pisani DF, Tulliez M et al. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. Blood. 2006;108:1652–60 [PubMed: 16670266]

132. Wernig G, Mercher T, Okabe R et al. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood. 2006;107:4274–81 [PubMed: 16478879]

133. Pikman Y, Lee BH, Mercher T et al. MPLW515L Is a Novel Somatic Activating Mutation in Myelofibrosis with Myeloid Metaplasia. PLoS Medicine. 2006;3:e270 [PubMed: 16834459]

134. Frey BM, Rafii S, Teterson M et al. Adenovector-mediated expression of human thrombopoietin cDNA in immune-compromised mice: insights into the pathophysiology of osteomyelofibrosis. J Immunol. 1998;160:691–9 [PubMed: 9551904]

135. kakumitsu H, Kamezaki K, Shimoda K et al. Transgenic mice overexpressing murine bopoietin develop myelofibrosis and osteosclerosis. Leuk Res. 2005;29:761–9 [PubMed: 15927672]

136. villeval JL, Cohen-Solal K, Tulliez M et al. High thrombopoietin production by hematopoietic cells induces a fatal myeloproliferative syndrome in mice. Blood. 1997;90:4369–83 [PubMed: 9373248]

137. Ulich TR, del Castillo J, Senaldi G et al. Systemic hematologic effects of PEG- rHuMGDF-induced megakaryocyte hyperplasia in mice. Blood. 1996;87:5006–15 [PubMed: 8652813]

- GATA1 expression is both necessary and sufficient for terminal maturation of erythroid cells and megakaryocytes.
- The expression of GATA1 in hematopoietic cells is regulated both at the transcription and mRNA translation level.
- GATA1 deficiency is a driving cause for numerous inherited and acquired diseases.
- GATA1 deficiency is the result not only of genetic mutations, but also of hypomorphic alterations of the efficiency of GATA1 mRNA translation
- A reduction in GATA1 protein expression is likely a key driver of the fibrotic process in PMF.
- Drugs targeting *GATA1* mRNA translation may represent a new frontier for the treatment of inherited and acquired human disorders such as Diamond Blackfan Anemia and PMF.

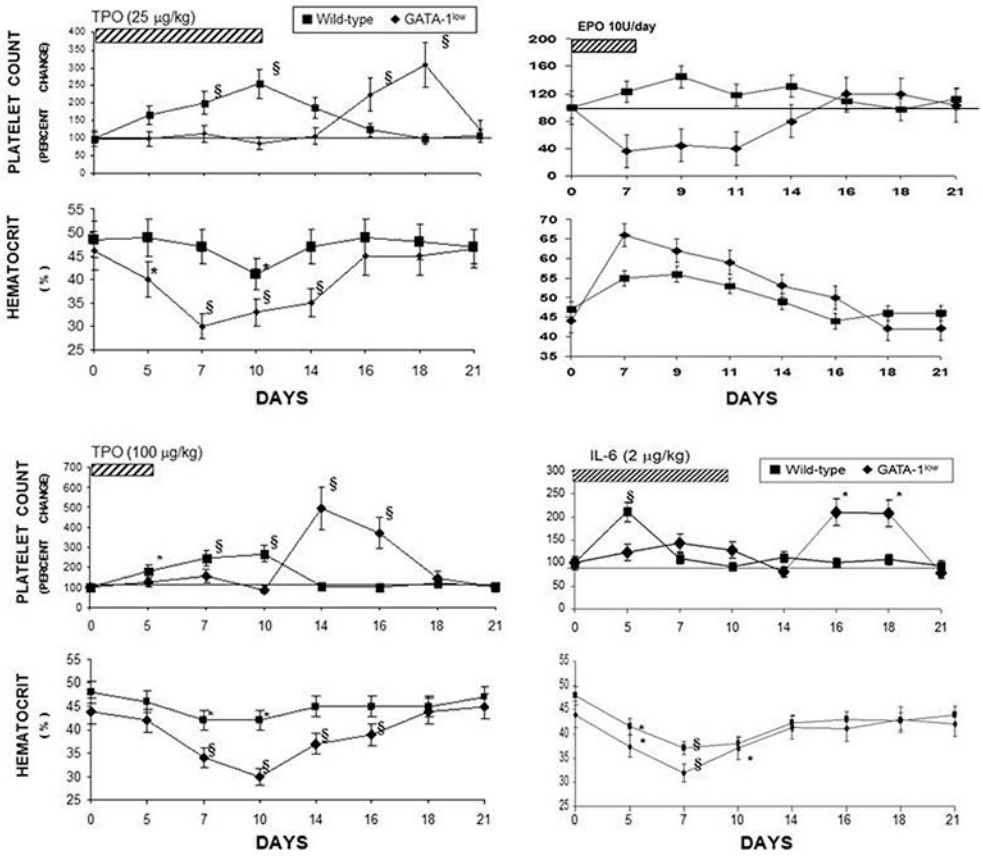

Figure 1. *Gata1*^{low} mice respond to treatment with growth factors which increase platelet counts and the hematocrit in the blood although the response occurs with a kinetics partially different from that observed with wild-type littermates.

The panels on the left present changes in platelet counts and hematocrit occurring over the course of 21 days in wild- type (black squares) and *Gata1*^{low} littermates treated with 25 (top) or 100 (bottom μg/Kg of body weight for the duration indicated by the horizontal bars while the panels on the right indicate changes occurring in response to EPO (10 U/mouse/Day, top) or IL-6 (2 μg/Kg, bottom). Results with 100 μg/Kg of TPO and with EPO are published by permission from Vannucchi et al., 2001 and 2005a [77,92]. Results with 25 μg/Kg of TPO and with IL-6 are instead not published and were obtained in collaboration with Dr. Vannucchi at the same time than the published observations.

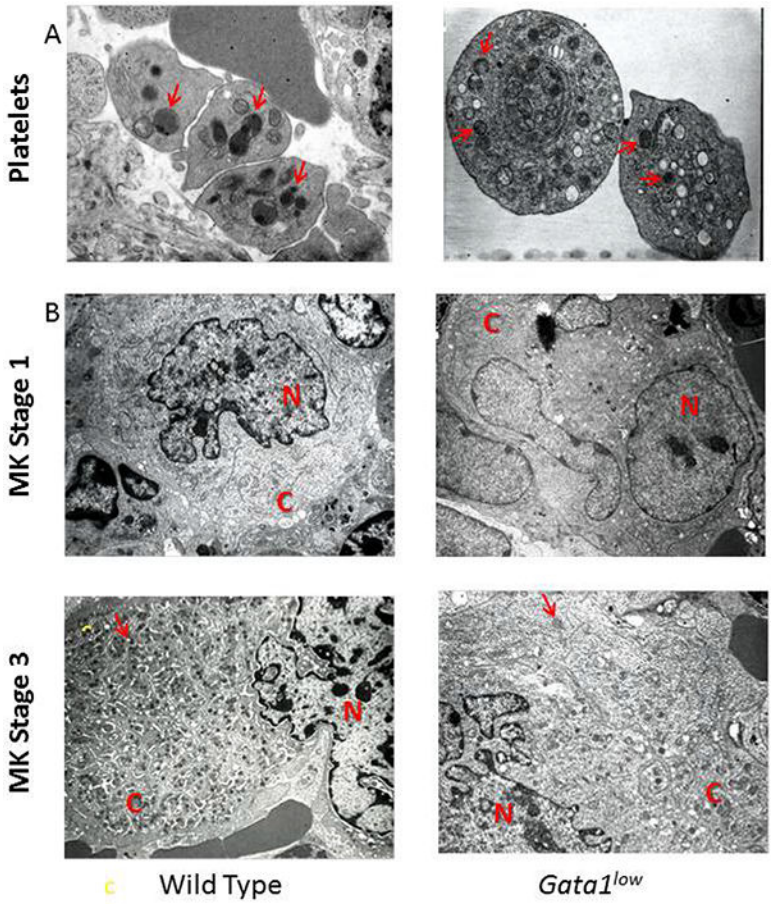

Figure 2. Electron microscopy analyses of representative platelets (A) and immature (stage 1) and mature (stage 3) megakaryocytes (MK) (B) from the bone marrow of wild-type and *Gata1*^{low} littermates.

A) Platelets from *Gata1*^{low} are larger than normal and their cytoplasm contains rudiments of the endoplasmic reticulum and fewer smaller and less electron dense granules (compare the electron density of the representative granules indicated by the red arrows in the left and right panels). B) Immature megakaryocytes from *Gata1*^{low} are not morphologically different from the corresponding wild type cells (upper panels). However, megakaryocytes with the typical morphology of mature cells (cytoplasm with well developed demarcation membrane system and well delineated platelet territories, the arrow on the bottom panel on the left) are never detected in the bone marrow or spleen from ***Gata1*^{low}** mice. The most mature megakaryocytes detected in organs from the mutants contain a rudimental demarcation membrane system and platelet territories with reduced electron density (the arrow on the bottom panel on the right). N=nucleus; C=cytoplasm. B Original magnifications 30000× in A and 3000× in B. Reproduced by permission from Centurioni et al., 2004 [83].

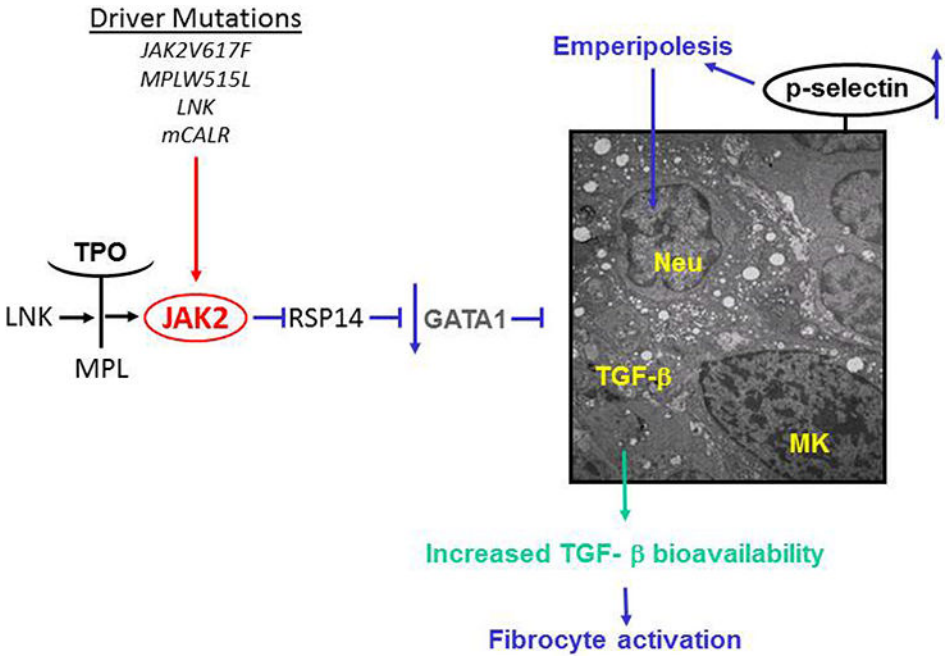

Figure 3. A model for the pathogenesis of primary myelofibrosis based on results obtained in animal models.

All the driver mutations associated with MPN, increased *TPO, MPL, JAK2V617F, CALR* or *LNK* activate the JAK/STAT pathway. Calreticulin (CALR) binds to MPL and induces constitutive activation of JAK/STAT. Lymphocyte linker (LNK) protein, a member of an adaptor protein family, is required to express MPL on the cell surface [124]. Only in PMF patients, however, activation of JAK/STAT signaling results, by a mechanism stillvpoorly understood, in a RPS14 deficiency which leads to reduced *GATA1* mRNA translation in megakaryocytes. Insufficient GATAI content retards megakaryocyte maturation increasing expression of p-selectin on their cell surface and triggering a process of pathological emperipolesis of neutrophils which lead to death of megakaryocytes by para-apoptosis. Dead megakaryocytes release TGF-ß in the microenvironment increasing the bioavailability of this growth factor. TGF-ß is then responsible to activate fibrocytes inducing bone marrow fibrosis.

Table 1.

Comparison of the changes in *Gata1* and *Gata2* mRNA occurring during the progression of erythroid and megakaryocyte differentiation and how these changes are affected by deletion of the HS1 enhancer in adult *Gata1*^{low} mice.

Mouse	Gene	CMP	MEP	Pro-E/Poly-E	Ortho-E	MK
Wild Type	***Gata1*** $2^{-\Delta Ct}$ (Fold Change)*	0.03 (1)	0.13 (4.3)	1.64 (54.7)	0.56 (18.7)	0.015 (0.5)
	Gata2 $2^{-\Delta Ct}$ (Fold Change)*	0.04 (1)	0.1 (2.5)	0.001 (0.025)	0.011 (0.28)	0.038 (0.95)
	Gata1/Gata2		1.3			
Gata1^{low}	***Gata1*** $2^{-\Delta Ct}$ (Fold Change)* [Fold Change]**	0.003 (1) [0.11]	0.05 (16.7) [0.38]	0.64 (213) [0.39]	0.19 (63) [0.01]	0.02 (0.66) [1.3]
	Gata2 $2^{-\Delta Ct}$ (Fold Change)* [Fold Change]**	0.03 (1) [0.75]	0.08 (2.6) [0.8]	0.003 (0.03) [3]	0.017 (3.3) [1.5]	0.06 (2) [1.6]
	Gata1/Gata2					

*with respect to the corresponding CMP.

**with respect to the corresponding wild-type populations.

#positive and negative fold changes are indicated in red and green fonts respectively. These results have been elaborated from primary data published previously [63,67,105].

Table 2.

Blood cell counts in littermates wild-type and hypomorphic at the *Gata1* locus.

Differences statistically different ($P<0.05$ by Anova) are in bold. Values observed in *Gata1*^{low} mice greater and lower than those observed in wild-type mice are in red and green fonts respectively. These results were partially published before [62,66].

Blood Cells Counts		
	Wild-type	**Gata1^{low}**
Erythrocytes(×10^6/mL)	8.78 ± 0.38	
Hemoglobin (gr/dL)	14.5 ± 0.7	12.13 ± 0.70
Hematocrit (%)	39.77 ± 1.80	
Mean corpuscolate volume (MCV) (10^{-15} L/RBC)	45.28 ± 0.58	48.49 ± 043
Mean cell hemoglobin (MCH) (10^{-12} g/RBC)	16.5 ± 0.26	16.98 ± 0.35
RDW (Red blood cell distribution width) (%)	16.59 ± 0.27	
Reticulocytes (%)	ND	
Platelets (}10^3/pL)	788.1 ± 69.27	
Mean platelet volume (MPV)(10^{-15} L/Platelet)	7.75 ± 0.08	
Platelet distribustion width (PDW) (%)	32.0 ± 4.2	
White blood cells (x10^3/pL)	5.63 ± 0.61	
White Blood Cells Composition (%)		
Myelocytes	0	0
Metamyelocytes	0	0
Immature neuthrophils	0	0.2 ± 0.2
Mature neutrophils	11.2 ± 1.5	
Lymphocytes	88.8 ± 1.5	
Monocytes	0	
Eosinophils	0	0
Basophils	0	0
White Blood Cells Composition (x10^3/μL)		
	0	0
	0	0
Immature neutrophils	0	
Mature neutrophils	0.56 ± 0.10	
Lymphocytes	4.98±0.54	
Monocytes	0	
Eosinophils	0	0
Basophils 0	0	0

[#]positive and negative fold changes are indicated in red and green fonts respectively. Results are expressed as Mean (±SD) of values observed with 10 mice (5 males and 5 females) per group. The mice were all 5–6-months old.

Table 3.

Total Plasma Levels (in ng) of selected growth factors in littermates wild-type and hypomorphic at the Gata1 locus.

Results represent the average concentrations detected in the blood from >8 mice per experimental group. Mice were 6–8 months old. Values statistically significant between wild-type and *Gata1*^{low} mice are in bold. Values greater and lower than controls are in red and green fonts respectively. The total levels of growth factors were obtained by multiplying their plasma concentrations [81,103,105] by the total blood volumes reported in Table 4

Growth Factor	Wild-Type	Gata1^{LOW}
	1.05	
Soluble SDF-1 (CXCL12)	23.4	
Platelet-associated SDF-1	72.0	
Bioactive TGF-β	4.50	6.5
Total TGF-β	31.50	

[#]positive and negative fold changes are indicated in red and green fonts respectively.

Table 4.

Total number of nucleated cells, stem/progenitor cells (Lin”Sca1^{+}Kit^{+}, LSK), common myeloid progenitors (CMP) and megakaryocyte erythroid progenitors (MEP) in hematopoietic organs of wild-type or hypomorphic (*Gata1*^{low} at the *Gata1* locus) littermates.

The number of erythroid cells at different stages of maturation (pro- /basophilic, Pro-E/Baso-E, polychromatophilic, Poly-E and orthochromatic, Ortho-E erythroblasts) and of megakaryocytes (MK) are also reported. All cell numbers are provided per 10^6.

Organ	Nucleated Cells	LSK	CMP	MEP	Pro/Baso-E	Poly-E	Ortho-E	MK
Blood								
Wild-type (3 mL)	35.2	<0.01	–	–	–	–	–	–
Gata1^{low} (2.7 mL)	14.9	0.3						
Fold Change	0.42[#]	>30						
Bone Marrow								
Wild-type	762	14.5	13.8	8.1	71.6	118.9	56.4	45.7
Gata1^{low}	297	4.5	4.6	1.7	13.1	5.5	22.9	68.3
Fold Change	0.39	0.31	0.33	0.21	0.18	0.05	0.41	1.50
Spleen								
Wild-type	114	2.9	bd	bd	7.3	5.5	11.7	21.7
Gata1^{low}	623	8.7	2.6	2.4	15.6	8.1	63.5	39.2
Fold Change	5.47	3.0	>100[*]	>100	2.14	1.47	5.42	1.81
Total								
Wild-type	911.2	17.3	13.8	8.1	78.9	124.4	68.1	67.4
Gata1^{low}	934.9	13.5	7.2	4.1	28.7	13.6	86.4	107.5
Fold Change	1.03	0.78	0.80	0.51	0.36	0.11	1.27	1.6

[#] positive and negative fold changes are indicated in red and green fonts respectively.

[*] fold change was calculated assuming a limit of detection of 0.01. Similar data were reported previously [63,67,105].

Table 5.

Summary of currently available mouse models for myelofibrosis generated by introducing driver mutations found in patients in the mouse genome *(JAK2V617F* indicates the human mutation whie *Jak2*^{V617F} is used for the mouse models).

Model	Time	Main features	References
Jak2$^{V617F/+}$ *Ezh2*$^{-/-}$ mice (Conditional *Jak2*^{V617F} knock- in, *Ezh2* floxed (*Ezh2*$^{fl/fl}$) and MxCre)	16, 24 weeks	Deletion of EZH2 inhibited erythropoiesis and promoted megakaryopoiesis in *Jak2*^{V617F} knock-in mice. Extensive fibrosis was found in the bone marrow and spleens of *Jak2*$^{VF/+}$ *Ezh2*$^{-/-}$ mice at 24 weeks after pI-pC induction, whereas *Jak2*$^{VF/+}$ mice exhibited very little or no fibrosis at this stage.	[125,126]
Bone marrow transplantation: CALRdel52 expressing bone marrow cells (By retroviruses carrying CALRdel52 cDNA)	6 months	CALRdel52 transplants showed a rapid and remarkably increased platelet counts. After 6 months, CALRdel52 expressing mice developed marked thrombocytosis and then progressed to a condition similar to myelofibrosis.	[127] ASH abstract
Jak2^{V617F} mice (Conditional *Jak2*^{V6l7F} knock-in and MxCre)	24 weeks	Mild fibrosis can be detected in the bone marrow of older heterozygous *Jak2*^{V617F} mice (24 weeks after induction). Pronounced fibrosis of the white pulp can be found in the spleens of both heterozygous and homozygous *Jak2*^{V617F} mice. Spleens of homozygous *Jak2*^{V6l7F} mice showed increased reticulin fibrosis in the red pulp compared with that of heterozygous mice.	[128,129]
Bone marrow transplantation: JAKV617F expressing bone marrow cells (By retroviral vector carrying murine *Jak2*^{V6l7F} cDNA)	1. 2, >4 months	Striking elevation in hematocrit, leukocytosis, megakaryocyte hyperplasia, extramedullary hematopoiesis resulting in splenomegaly, and reticulin fibrosis in the bone marrow.	[130–132]
Bone marrow transplantation: MPLW515L expressing bone marrow cells (By MSCV retroviral vector carrying human *MPL*^{W515} cDNA)	2–3 weeks	MPLW515L-transplants developed lethal myeloproliferative disorders with marked thrombocytosis and leukocytosis. Those mice also showed splenomegaly, hepatomegaly and reticulin fibrosis in bone marrow.	[133]
TPO^{high} (By adenoviral vectors carrying human *TPO* cDNA, 10^9 PFU in a volume of 100 μl, single i.p. administration on day 0)	12 weeks	SCID (severe combined immunodeficient; T and B cell defect, diminished CTL, low to absent Ab production) mice developed thrombocytosis, leukocytosis, and marked megakaryocyte hyperplasia in the spleen and bone marrow with a progressive myelofibrosis and osteosclerosis.	[108,134]
TPO^{high} mice (By expressing full-length murine TPO under the control of the mouse H chain enhancer/ promoter)	9–12 months	Mild leukocytosis (~2-fold), anemia (~10% decrease in hematocrit), thrombocytosis (3-fold), and increase of plasma TPO level (3.5 ±1.5 ng/ml) were associated with bone marrow fibrosis starting at 9 months.	[135]
Gata1^{low} or *Gata1*$^{neo\Delta HS}$ mice (Delete DNase I-hypersensitive region by replacing ~8 kb of upstream sequences, including the IT and FIS regions, with a PGK-NeoR cassette flanked by loxP sites)	11 months	Mutant mice got selective loss of megakaryocyte GATAI expression. They had markedly reduced platelet numbers (~15% of normal), associated with the number of megakaryocytes in hematopoietic tissues was greatly increased. Anemia was associated with myelofibrosis in the marrow and in the spleen.	[62]
Bone marrow transplantation: TPO expressing bone marrow cells (By retroviral vectors carrying murine Tpo cDNA)	2–3 months	7–9 weeks post-transplant, features were a massive hyperplasia of megakaryocytes and granulocytes in the spleen and bone marrow and a hypoplasia of erythroblasts in bone marrow. From 10 weeks post-transplant, WBC, platelets, and red blood cell numbers declined dramatically. Deposits of reticulin fibers were observed 3 months after transplantation.	[136]
TPO^{high} (Administration of PEG-rh-MDGF)	2 weeks	Immunocompetent BALB/c mice were injected daily for 14 days with increasing doses of PEG-rh-MDGF from 10 to 500 μg/kg. All animals developed dose-dependent thrombocytosis, moderate leukocytosis, anemia, splenomegaly, and extramedullary hematopoiesis. Bone marrow fibrosis was observed at the highest dose injected, which reversed rapidly when the injections were stopped.	[108,137]