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Novel targets to cure primary myelofibrosis from studies on *Gata1*^{low} mice

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Abstract

In 2002, we discovered that mice carrying the hypomorphic *Gata1*^{low} mutation that reduces expression of the transcription factor GATA1 in megakaryocytes (*Gata1*^{low} mice) develop myelofibrosis, a phenotype that recapitulates the features of primary myelofibrosis (PMF), the most severe of the Philadelphia-negative myeloproliferative neoplasms (MPNs). At that time, this discovery had a great impact on the field because mutations driving the development of PMF had yet to be discovered. Later studies identified that PMF, as the others MPNs, is associated with mutations activating the thrombopoietin/JAK2 axis raising great hope that JAK inhibitors may be effective to treat the disease. Unfortunately, ruxolitinib, the JAK1/2 inhibitor approved by FDA and EMEA for PMF, ameliorates symptoms but does not improve the natural course of the disease, and the cure of PMF is still an unmet clinical need. Although *GATA1* is not mutated in PMF, reduced GATA1 content in megakaryocytes as a consequence of ribosomal deficiency is a hallmark of myelofibrosis (both in humans and mouse models) and, in fact, a driving event in the disease. Conversely, mice carrying the hypomorphic *Gata1*^{low} mutation express an activated TPO/JAK2 pathway and partially respond to JAK inhibitors in a fashion similar to PMF patients (reduction of spleen size but limited improvement of the natural history of the disease). These observations cross-validated *Gata1*^{low} mice as a bona fide animal model for PMF and prompted the use of this model to identify abnormalities that might be targeted to cure the disease. We will summarize here data generated in *Gata1*^{low} mice indicating that the TGF-β/P-selectin axis is abnormal in PMF and represents a novel target for its treatment.

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AUTHOR CONTRIBUTION

All the authors have contributed to writing the paper and concur with its content.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

Keywords

cancer supporting microenvironment; *Gata1*; hematopoietic stem cells; megakaryocytes; primary myelofibrosis; P-selectin; TGF-β

1 | INTRODUCTION

Primary myelofibrosis (PMF) is the most severe of the Philadelphia-negative myeloproliferative neoplasms (MPNs), which include polycythemia vera (PV) and essential thrombocytopenia (ET).[1,2] As all the other MPNs, PMF is a clonal disorder of the hematopoietic stem cells (HSC),[3,4] which usually has sporadic occurrence, although cases of familiar PMF have been occasionally reported.[1,2] PMF is manifested with thrombocytopenia and anemia associated with the presence of platelets larger than normal (megathrombocytes) and red blood cells with tear drop shape (tear drop poikilocytes) in the blood.[1,2] The bone marrow of PMF patients presents distinctive microenvironment abnormalities with excessive deposition of extracellular matrix (fibrosis), increased proliferation of osteoblasts (osteosclerosis) and endothelial cells (neoangiogenesis). These abnormalities lead to hematopoietic failure in bone marrow, increased stem/progenitor cell trafficking, and development of hematopoiesis in extramedullary sites, mostly the spleen. The disease eventually evolves in leukemia.[1,2] The complexity of the PMF phenotype makes unlikely that the disease is driven by the mutation of a single gene. Furthermore, the presence of thrombocytopenia and anemia, two traits regulated by the transcription factor GATA1,[5] suggests that in some way the driver mutation(s) impair GATA1 functions. In spite of the great progresses made in recent years in our understanding of the genetic lesions driving PMF, the etiology of PMF is far to be completely understood and is the subject of numerous investigations. These investigations are prompted by the belief that, besides their therapeutic implications for PMF, studies on the etiology of MPN have the potential to share light also on the complex interactions between stem cell and microenvironmental abnormalities that support progression to the metastatic phase in other cancers and on mechanisms underlying development of the fibrosis preceding the fatal failure of other organs.

This review will summarize the role played by megakaryocyte abnormalities induced by hypomorphic GATA1 content on the etiology of PMF, with the hope that this information will be useful to understand the pathogenesis of other diseases as well.

2 | THE MUTATION LANDSCAPE OF PMF DOES NOT INCLUDE *Gata1*

In 2005, four groups identified that MPNs are associated with the gain-of-function *JAK2*V617F mutation in the gene encoding the Janus Kinase-2 (*JAK2*, >95% of PV and 50–60% of ET and PMF).[6–9] JAK2 is a tyrosine kinase that initiate the signaling from all the hematopoietic superfamily receptors, including the receptors for erythropoietin (EPO), thrombopoietin (TPO), and granulocyte-colony stimulating factor (G-SCF),[10] and that controls the proliferation of normal hematopoietic cells of many lineages. It is not surprising, therefore, that its constitutive activation may drive hyperproliferation of several hematopoietic lineages inducing diseases associated with excessive red blood cell production, PV, or excessive production of megakaryocytes with (PMF) or without (ET) a

block in platelet production. Since the discovery of *JAK2*V617F, the mutation landscape of MPNs has been refined with the identification of additional mutations in the JAK2/MPL axis in those patients who lack canonical JAK2 mutations.[10,11] Furthermore, PMF patients may present gain-of-function mutations in additional genes involved in epigenetic and splicing regulation and in induction of apoptosis.[10,11] Since these mutations are found across all the hematopoietic malignancies and their acquisition may either precede or follow that of the JAK2/MPL mutations, they are thought to drive the evolution of PMF to leukemia. In spite of the great progress made on the knowledge on the genetic basis of MPNs, the mechanism(s) that decides how the same JAK2/MPL mutation may induce hyperproliferation of either the erythroid (PV) or the megakaryocyte lineage (ET and PMF) is still far to be elucidated. Studies in vitro and in mouse models suggest that the erythroid or megakaryocyte phenotype is determined by the levels of activation of STAT5, the signaling element immediately downstream to JAK2V617F with high and low levels of STAT5 activation driving PV or ET, respectively.[12–15] In addition, the mechanisms determining the block in maturation of the hyperproliferating megakaryocytes in PMF may be represented by a RSP14 ribosomopathy (reviewed in Reference 16).

3 | PMF MEGAKARYOCYTES ARE HYPOMORPHIC FOR *Gata1*

The hypomorphic *Gata1*^{low} mutation, which specifically deletes the first hypersensitive site (HS1) upstream to the gene, was developed in 1997 in the Stuart Orkin laboratory.[17] At that time, the establishment of this mutation, and of the similar *Gata1*$^{0.5}$ mutation developed by the Iamamoto laboratory,[15] represented a paradigm shift in our approach to study the genetic control of protein function. In fact, starting from these mutations, the focus of these studies switched from determining how the function of a protein was controlled by gene coding sequences on how it was instead controlled by noncoding sequences that regulate its levels of expression. Studies on *Gata1*^{low} and *Gata1*$^{0.5}$ mice have identified the first lineage-specific regulatory sequences and assessed that erythroid and megakaryocyte differentiation is assured by an appropriate protein concentration window inside the cells.[15,17,18] The mutation was originally established in the C57BL/6 background, where it induces a high (>90%) perinatal mortality due to severe anemia.[17] In the CD1 background, however, the mutation is no longer perinatally lethal,[19] and the mice have an apparently normal life span because, by recruiting within few weeks, the spleen as an extramedullary erythropoietic site expresses normal hematocrit levels from 1 to 18 months of age.[19] The splenic erythropoiesis of *Gata1*^{low} mice does not exhaust the response to “erythroid stress” since these mice are still capable of increasing hematocrit levels when treated with exogenous EPO and recover from hemolytic anemia induced by phenylhydrazine.[19] It is thought that the splenic erythropoiesis occurring in *Gata1*^{low} mice at steady state is sustained by a pathway different from that activated during canonical “stress erythropoiesis” because it does not involve the glucocorticoid/BMP pathway[20] but rather hyperactivation of the TPO pathway.[21,22] Hyperactivation of TPO signaling generates in the spleen of *Gata1*^{low} mice a unique precursor cell that remains bipotent for the erythroid and megakaryocyte pathway until the very end of the maturation process.[23,24] These precursor cells are similar to those observed in vitro when human CD34+ cells are cultured with a recombinant hyperactive TPO mimetic.[25]

Proof that almost all the red blood cells circulating in the blood of *Gata1*^{low} mice are generated by the spleen is provided by the observation that splenectomy induces death of the mice by profound anemia within 2 weeks.[26] In addition, in *Gata1*^{low} mice almost all the hematopoietic stem cells are present in their spleen.[27]

The fact that the CD1 *Gata1*^{low} mice do not die at birth allowed the identification that GATA1 also regulates terminal maturation of mast cells[28] and of dendritic cells.[29] In the latter cases, the mutation increases the cell proliferation rates but impairs their ability to present antigens in response to lipopolysaccharides, suggesting that *Gata1*^{low} mice also have impaired ability to activate T cells.

The megakaryocyte abnormalities expressed by *Gata1*^{low} mice include increased proliferation and delayed maturation and are extremely similar to those found in PMF (Figure 1).[30–32] It is therefore not surprising that *Gata1*^{low} mice develop myelofibrosis with robust levels of collagen fibers in the bone marrow with age.[33,34]

The similarity between the megakaryocyte abnormalities observed in *Gata1*^{low} mice and PMF patients suggests that they are determined by the same mechanism(s). In fact, also megakaryocytes from PMF patients are hypomorphic for GATA1 because, in spite of normal levels of GATA1 mRNA,[35] they express a RSP14 signature, which impairs the translation of *GATA1* mRNA.[36] The list of the RSP14-dependent genes included in the GeneSet database and how the expression of these genes is altered in *Gata1*^{low} mice are included in Table S1. In addition, although at birth *Gata1*^{low} megakaryocytes express, as expected, reduced levels of *Gata1* mRNA (Figure 1a), in adult mice the mutation reduces the levels of *Gata1* mRNA mostly in stem/progenitor cells, altering the GATA1/GATA2 switch that controls the proliferation of hematopoietic progenitor cells[37] favoring their expansion[38] but not in the megakaryocytes (Figure 1b). However, as in the case of PMF patients, the low GATA1 content of these cells (Figure 1c) is probably the result of a RSP14 ribosomopathy that reduces the numbers of ribosomes in these cells (Figure 1d) impairing the translation of *Gata1* mRNA.[22] Also in the case of *Gata1*^{low} mice, the RSP14 signature is induced by a hyperactive MPL/JAK2 pathway (Table 1).[22]

The discovery of the mutations that drive the development of MPNs prompted the development of mutation-specific mouse models (reviewed in Reference 59). These mutants rapidly develop a PV and/or ET phenotype, which eventually evolves in myelofibrosis, and are considered models for secondary myelofibrosis. The value of these mutants for PMF, however, is limited by the fact that their bone marrow contains high levels of reticulinic fibers (fibers with low levels of collagen polymerization detected at the early stage of the disease) but it is unclear if they ever develop the collagen fibers developed when the disease progress to myelofibrosis. These limitations and the similarities existing between the activation of the MPL/JAK2 pathway and the RSP14 ribosomapathy observed in PMF patients and *Gata1*^{low} mice finally established *Gata1*^{low} mutants as bona fide model for PMF.

3.1 | Myelofibrosis is a disease of the microenvironment sustained by the abnormal megakaryocytes

In 2007, two papers by Walkey et al. established that mutations altering the microenvironment are necessary and sufficient to induce an MPN phenotype in mice, suggesting that the establishment of MPN does not require abnormal hematopoietic stem cells.[60,61] More recently, two independent studies have refined this concept by demonstrating that expression of *JAK2*V617F in megakaryocytes, which are important regulators of the microenvironment,[62] is necessary and sufficient to induce myelofibrosis in mice.[63,64] Together, these observations suggest that PMF is driven by products produced by the abnormal megakaryocytes that alters the stem cell-supporting functions of the microenvironment and raise hope that the disease may be cured by targeting these products.

The hypothesis that megakaryocyte abnormalities drive the development of PMF is not new and has been supported by extensive morphological data. The presence of distinctive megakaryocyte abnormalities characterized by hyperproliferation with retarded maturation was the first cellular hallmark for PMF to be identified.[32] In addition to be present in high number as immature cell clusters, an indication of hyperproliferation, the megakaryocytes present in the bone marrow of PMF patients contain high levels of the growth factor TGF-β[44,45] and are embedded by high numbers of neutrophils entrapped in their cytoplasm by a process of pathological emperipolesis.[31,65] It is important to mention that TGF-β is one of the growth factors responsible for the epithelial-mesenchymal transition, which induces the formation of activated fibrocytes suggested by Robert Weinberg to be responsible for fibrosis and disease progression in cancer.[66] Furthermore, the bone marrow of PMF patients contains high numbers of naked nuclei with heavily condensed chromatin, which are probably the reminiscence of megakaryocytes that died by an immune-mediated process defined para-apoptosis.[31,49] These morphological observations suggest that the microenvironment surrounding the para-apoptotic megakaryocytes is enriched for growth factors produced by these cells and activated by the neutrophil proteases.

Also the megakaryocytes from *Gata1*^{low} mice remain immature and are subjected to death by para-apoptosis (Figure 2). In addition, they express high levels of TGF-β and P-selectin, the adhesion receptors that mediates their interaction with neutrophils (Figure 3). These observations lead to the pathobiological model for myelofibrosis summarized in Figure 4, which hypothesizes that the disease is sustained by progressive TGF-β accumulation in the microenvironment triggered by pathological P-selectin-dependent emperipolesis between megakaryocytes and neutrophils.

This model was tested by experiments indicating that chemical inhibition of TGF-β signaling[44] or genetic ablation of *P-selectin*[27] completely restores megakaryocyte maturation (Figure 2) and rescues the myelofibrotic phenotype of *Gata1*^{low} mice. These observations support the therapeutic hypothesis that cure of myelofibrosis requires targeting the abnormalities of both the malignant stem cells and of their supporting microenvironment.[67] This hypothesis is testable since both TGF-β and P-selectin are druggable by products developed for clinical use in other diseases. TGF-β may be inhibited either by galunisertib, a small molecule that inhibits AKL5, the first element of the TGF-β receptor 1 signaling[68] or by the TGF-β receptor trap AVID200 developed by Forbious.[69] P-selectin may instead be

inhibited by crizanlizumab, an antibody developed by Novartis, which has been shown effective to prevent pain crisis in Sickle Cell Diseases.[67] Phases 1–2 clinical trials with AVID200 (NCT03094169, open for accrual) and Crizanlizumab (NCT not available as yet) in combination with ruxolitinib are in progress.

The identification of biomarkers to evaluate disease progression or response to therapy is another important facet in the process to address the clinical need of a disease. The comparison of the expression signature of bone marrow from *Gata1*^{low} mice and PMF patients identified common abnormalities in 12 genes of the noncanonical TGF-β signaling[67,70] which include Jun, JUNB, FOS, FOSB, HIPK2, ID1, GADD45b, BMP4, BMP7, EVI1, STAT1, and IL8.[50,71] Jun, JUNB, FOS, and FOSB are transcription factors involved in the formation of the complex AP-1 (activator protein 1), which regulate gene expression in myelomonocytic cells and recently suggested by the Weissman laboratory of be responsible to induce fibrosis in multiple organs, including in bone marrow.[72] Altered expression of HIPK2 (homeodomain-interacting protein kinase 2) has been implicated in the development of kidney fibrosis.[73] ID1 (DNA-binding protein inhibitor 1) and GADD45b (growth arrest and DNA-damage-inducible beta) are two genes implicated in the growth and progression of multiple cancers.[74,75] BMP4 and BMP7 (bone morphogenetic proteins 4 and 7) are among the growth factors that regulate bone and cartilage formation and may be responsible for the osteosclerosis expressed by *Gata1*^{low} mice[76] while EVI1 (ecotropic virus integration site 1 protein homolog) was among the first transcription factors involved in tumor development in mice which has been implicated in drug resistance in human leukemia.[77] On the other hand, the signal transducer and activator of transcription (STAT) 1 protein is another STAT family member downstream of JAK2 signaling and its activation has been implicated in development of myelofibrosis by the Skoda laboratory.[78] Last but not least, IL-8 (Interleukin 8) is a cytokine produced by many cells known to be overexpressed in PMF, but not in ET the levels of which may represent an infaust prognostic criteria in PMF.[79]

These genes are included in arrays currently under development to be used as diagnostic or prognostic tool in PMF.

Besides the TGF-β/P-selectin circuit discussed above, the profile of *Gata1*^{low} mice and PMF patients includes several additional abnormalities many of which are also druggable (Table 1). The preclinical studies which are in progress to validate their use as therapeutic targets for PMF are reviewed in Reference 16.

Last but not least, the expression signature of the bone marrow of *Gata1*^{low} mice includes the transcription factor c-Jun which is expressed at altered levels also in the fibrosis state of other organs,[72] raising hope that the same therapeutic strategy may be used to cure fibrosis in multiple organs. To test this hypothesis, we are currently investigating whether *Gata1*^{low} mice develop fibrosis in organs other than bone marrow.

4 | CONCLUSIONS

The interplay between hematopoietic stem cells and their supporting microenvironment in the regulation of hematopoiesis was originally identified thanks to mice carrying mutations at the steel and white locus. Steel mice carry mutations in the gene encoding stem cell factor, the stem cell specific growth factor and is expressed by the microenvironment. The white mice carry mutations in *c-Kit*, the gene encoding the receptor for stem cell factor expressed on the stem cells. The numerous studies on the effects exerted by mutations at these two single loci on adult hematopoiesis have greatly contributed to shape up our understanding of its regulation (reviewed in References 80–84). Studies on adult hematopoiesis in *Gata1*^{low} mice have now clarified an important facet of the interplay between the stem cells and their niche. They have put in the picture megakaryocytes as the progeny of the hematopoietic stem cells responsible to modulate the supporting functions of their niche in response to physiological hematopoietic perturbations. In the case of *Gata1*^{low} mice, however, chronic platelet deficiency activates a TPO/JAK2 axis, which sustains the maturation of abnormal megakaryocytes responsible for the activation of unique niches in the spleen that turn normal hematopoietic stem cells into malignant clones, inducing myelofibrosis. By putting megakaryocytes at the center of the myelofibrosis etiology, studies in *Gata1*^{low} mice provide proof-of-principle in animal models that PMF may be prevented and reversed by targeting products (TGF-β, P-selectin, and possibly others) released by megakaryocytes to regulate the hematopoietic stem cell niches.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ALK5	Anaplastic Lymphome Kinase 5
AP-1	Activator protein 1
BMP4–7	Bone Morphogenetic Protein 4–7
CD	Cluster of Differentiation
CMP	Common Myeloid Progenitor
CXCR4	C-X-C chemokine receptor type 4

DNA	DeoxyriboNucleic Acid
EMEA	European Medicines Evaluation Agency
EPO	Erythropoietin
ET	Essential Thrombocytopenia
EVI1	Ecotropic Virus Integration site 1 protein homolog
FcεRIα	High-affinity IgE receptor subunit α
FDA	Food and Drug Administration
FITC	Fluorescein isothiocyanate
FOS	transforming gene of Fibroblast OsteoSarcoma Virus
GADD45b	Growth Arrest and DNA Damage 45b
GATA1–2	GATA-binding factor 1–2
G-SCF	Granulocyte-Colony Stimulating Factor
HIPK2	Homeodomain-Interacting Protein Kinase 2
HS1	Hypersensitive Site 1
HSC	Hematopoietic Stem Cells
ID1	DNA-binding protein inhibitor
IL	Interleukin
JAK1–2	Just Another Kinase 1–2
JUN, ju-nana	Japanese word for seventeen
LIN	Lineage
LOX2	Lipoxygenase-2
Ly6c	Lymphocyte antigen 6 complex
MEP	Megakaryocytes-Erythroid Progenitors
MK	Megakaryocytes
MPL	Myeloproliferative Leukemia Protein
MPN	Myeloproliferative Neoplasm
OrthoE	Orthichromatic Erythroblasts
PE- Cy7	Phycoerythrin and Cyanine dye
PMF	Primary Myelofibrosis

PolyE	Polychromatic Erythroblasts
ProE	ProErythroblast
PV	Polycythemia Vera
RSP14	Ribosome Protein 14
RT-PCR	Reverse transcription polymerase chain reaction
SCA1	Stem cells antigen-1
SDF-1	Stromal cell-Derived Factor 1
STAT1–5	Signal Transducer and Activator of Transcription 1–5
TGF-β	Tumor/Trasforming Growth Factor β
TPO	Thrombopoietin
WT	Wild Type

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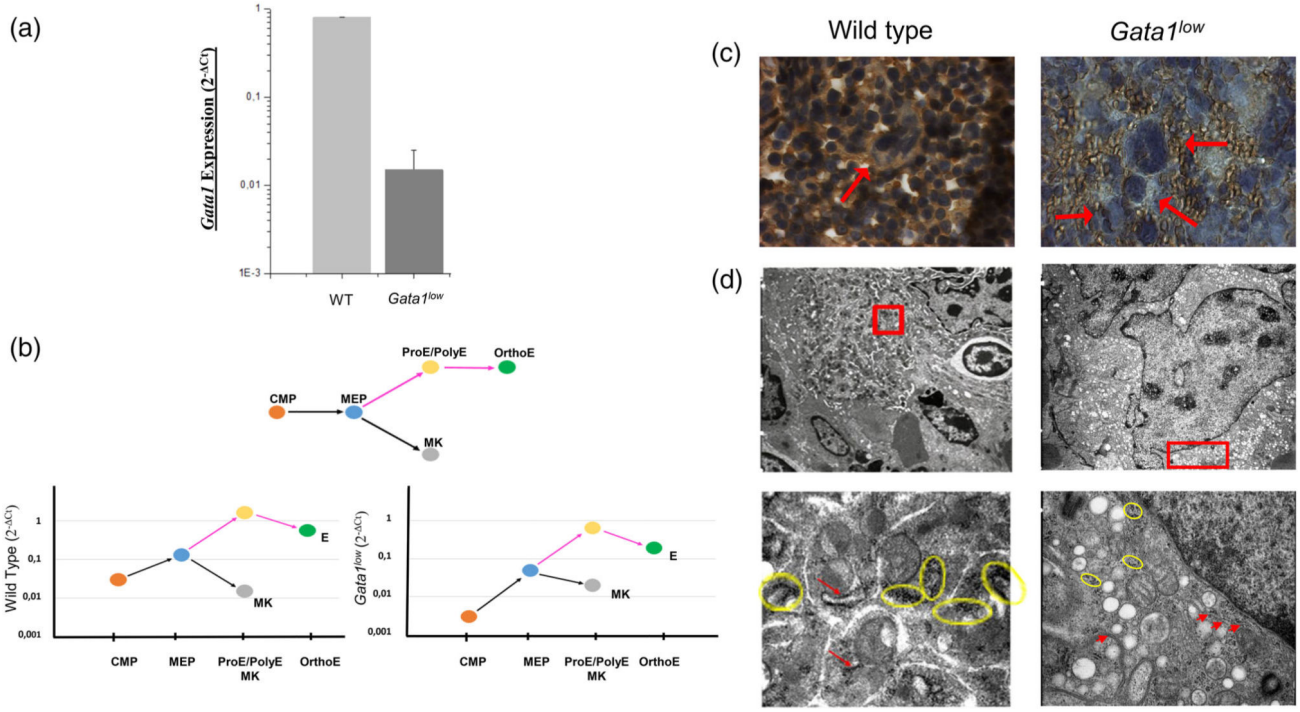

FIGURE 1.

Reduced frequency of ribosomes decreases the GATA1 content in the megakaryocytes from the spleen of *Gata1*^{low} mice in spite of normal levels of Gata1 mRNA. (a) Quantitative RT-PCR determinations of the levels of *Gata1* mRNA expressed by megakaryocytes prospectively isolated from the bone marrow of one-month old wild-type (WT) and *Gata1*^{low} littermates showing that *Gata1*^{low} megakaryocytes express reduced levels of *Gata1* mRNA. (b) Quantitative RT-PCR determinations of the levels of *Gata1* mRNA expressed by common myeloid progenitor cells (CMP, Lin^{-} Sca1^{-} Ly6c^{-} FcεRIα^{-} c-kit^{pos} CD34^{low} CD16/CD32^{+}), bipotent megakaryocyte-erythroid progenitor cells (MEP, Lin^{-} Sca1^{-} Ly6c^{-} FcεRIα^{-} c-kit^{pos} CD34^{low} CD16/CD32^{low}), early (proerythroblasts, ProE, and polychromatic erythroblasts, PolyE) and late (orthochromatic erythroblasts, OrthoE) erythroblasts and megakaryocytes (MK) prospectively isolated from the spleen of WT and *Gata1*^{low} littermates, as indicated. *Gata1*^{low} CMP, MEP and erythroid cells express levels of *Gata1* mRNA onefold lower than that expressed by the corresponding WT cells while the levels of *Gata1* mRNA in *Gata1*^{low} megakaryocytes is within normal ranges. (c) Immunostaining with a *Gata1* specific antibody of the spleen from WT and *Gata1*^{low} littermates. The nuclei of WT megakaryocytes, but not those from *Gata1*^{low} littermates, were readily stained by the antibody. Megakaryocytes are indicated by arrows. Magnification ×100. (d) Transmission electron microscopy of representative megakaryocytes, top panels, from WT and *Gata1*^{low} littermates and details of their perinuclear area (bottom panels). The detail of the *Gata1*^{low} megakaryocyte indicate the presence of a poorly developed rough endoplasmic reticulum with reduced numbers of polysomes (indicated by the yellow circles). Magnifications ×4,400 and ×30,000 in the top and bottom panels, respectively. Yellow Circles indicated polyribosomes and red arrows show poorly developed rough endoplasmic reticulum. Similar results were published in References 22 and 39

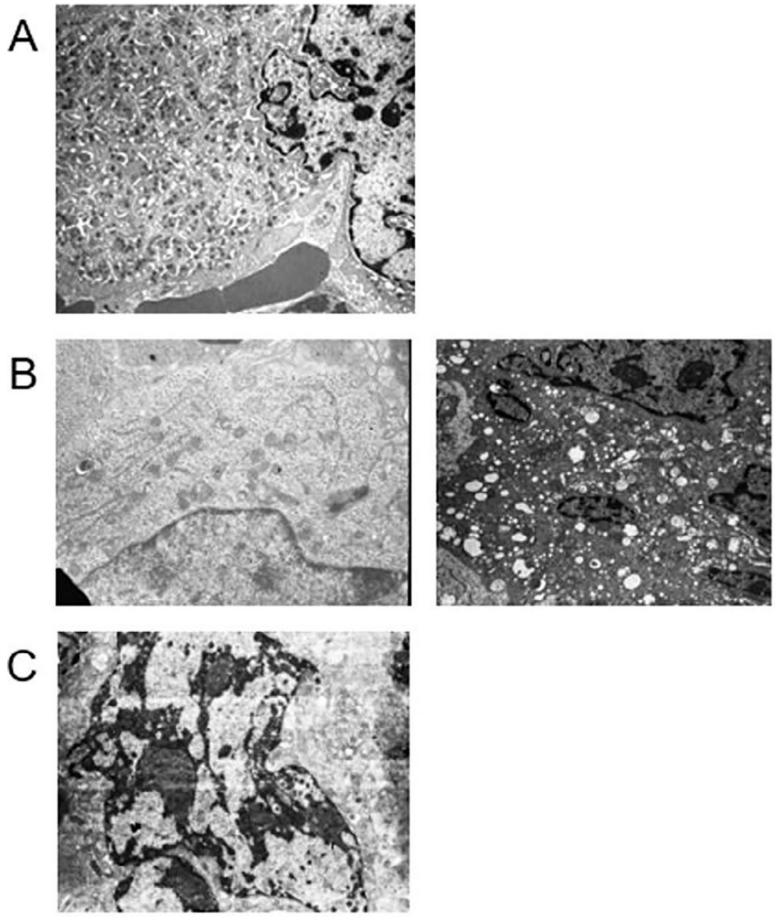

FIGURE 2.

Gata1^{low} megakaryocytes remain immature and the nuclei of some of them present a tightly condensed chromatin characteristic of para-apoptosis, an immune-mediated form of cell death probably induced by pathological neutrophil emperipolesis. (a) Electron microscopy analyses of a mature wild-type megakaryocyte with properly developed demarcation membrane system and delineated platelet territories. (b) Electron microscopy analyses of one representative immature (left panel) and one representative para-apoptotic (right panel) *Gata1*^{low} megakaryocyte. (c) Electron microscopy analyses of a representative mature megakaryocyte from a *Gata1*^{low} mouse treated with the TGF-β inhibitor SB431542. Magnification ×4,400. Similar results were published in References 31 and 45

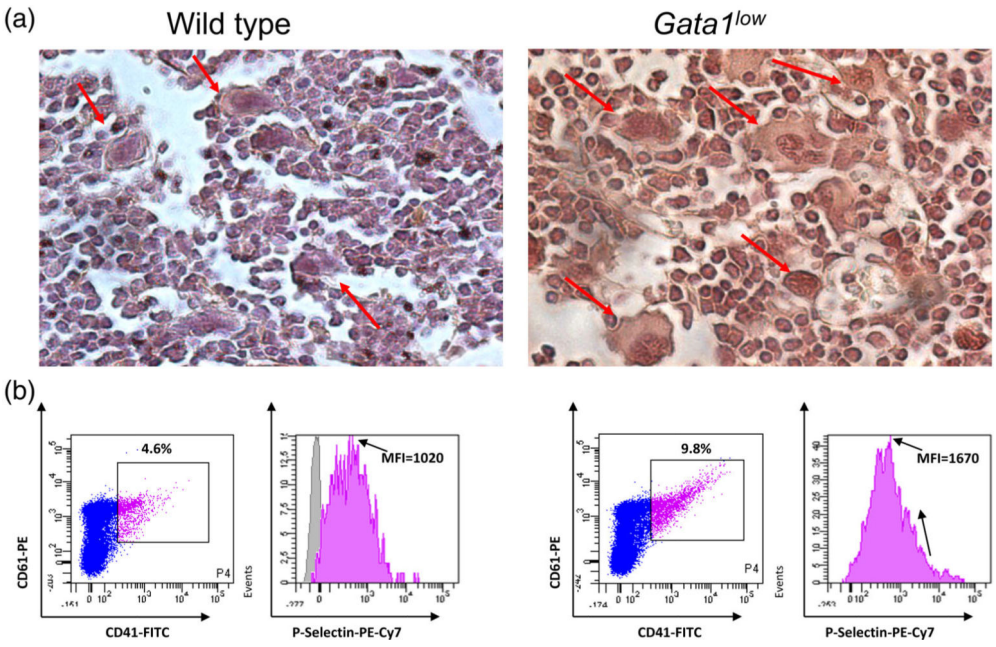

FIGURE 3.

Gata1^{low} megakaryocytes express levels of TGF-β and of P-selectin greater than normal. (a) TGF-β-specific immunohistochemistry of bone marrow sections from representative wild-type and *Gata1*^{low} littermates, as indicated. To be noted the intense immunostaining of the *Gata1*^{low} megakaryocytes. Magnification ×40. Megakaryocytes are indicated by arrows. Similar results were published in Reference 44. (b) Flow cytometry analyses for the expression of P-selectin by megakaryocytes from wild-type and *Gata1*^{low} littermates. Megakaryocytes were identified on the basis of CD41/CD61 markers, as indicated. Expression of high levels of P-selectin in *Gata1*^{low} megakaryocytes was also detected by electron microscopy[31]

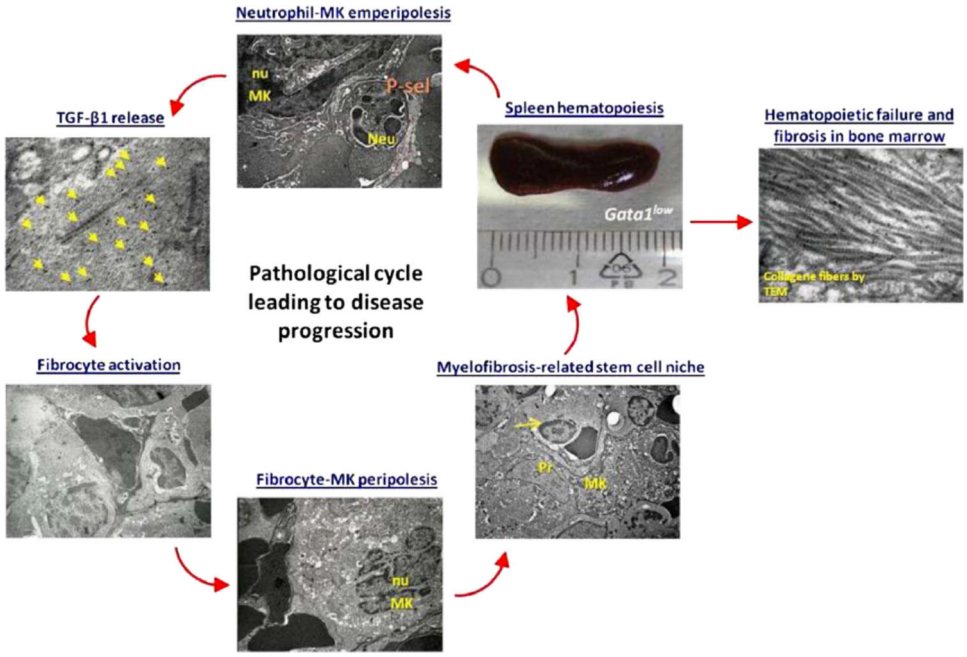

FIGURE 4.

An outline of the P-selectin/TGF-β circuit leading to disease progression in the *Gata1*^{low} mouse model of myelofibrosis. We propose that in *Gata1*^{low} mice, hematopoiesis in the spleen is sustained by a circuit between P-selectin and TGF-β and contributes to disease progression. This circuit is triggered by the abnormal expression of P-selectin on megakaryocytes (MK) that leads to neutrophilmegakaryocyte emperipolesis, increasing TGF-β content in the surrounding microenvironment and resulting in fibrocyte activation. Activated fibrocytes establish, possibly through P-selectin, peripolesis with MK forming "myelofibrosis-related stem cell niches" that sustain proliferation of the malignant stem cells in spleen generating more megakaryocytes and more neutrophils, establishing an amplification loop that contributes to disease progression. This loop may also determine hematopoietic failure and fibrosis in bone marrow. Neu, neutrophil; nu, nucleus; Pr, fibrocyte protrusion; yellow arrow, immature hematopoietic cell; yellow arrowheads, TGF-β-immuno, gold particles. The scale allows appreciation of the splenomegaly experienced by *Gata1*^{low} mice

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TABLE 1

Summary of the abnormalities expressed by primary myelofibrosis (PMF) patients that were first identified in *Gata1*^{low} mice and that represent potential druggable targets to cure the disease

	Abnormality	***Gata1*^{low} mice**	**PMF patients**
HSC	↑CXCR4	39	39–42
	↑Mobilization	39	43
Megakaryocytes	↑TGF-β	27, 44	44–47
	↑P-selectin	27	48
	↑LOX2	34	34
	↑Neutrophil emperipolesis	31	45
	↑Parapoptosis	31	49
Neutrophils	↑DNA trap	50	51
	↑Emperipolesis	31	45
Bone marrow microenvironment	↑TGF-β bioavailability	44	44
	↑Activated fibrocytes	27	52
	↑Collagen fibers	33, 35	1, 2
	↓Normal HSC	27, 44	53, 54
T cells	↓Activation	50	55, 56
Spleen microenvironment	↑Malignant HSC	27, 44	53, 54
	↑Megakaryocytes	33	1, 2
	↑Activated fibrocytes	27, 44	44
	↑Abnormal endothelial cells	33	57
	↑TGF-β bioavailability	44	44
Plasma/serum	↑TPO	22	-
	↑SDF-1	39	41, 58
	↑Bioactive TGF-β	44	56
	↑Total TGF-β	44	55, 56
	↑Antimitochondrial antibodies	-	50