GFI1B in Stem Cell Biology and Cancer

Anguita E1,*, Candel F.J.2, Chaparro A1 and Roldán-Etcheverry J.J.1

1Department of Hematology, Hospital Clinico San Carlos, IdISSC, Complutense University of Madrid, Spain
2Department of Microbiology, Hospital Clinico San Carlos, IdISSC, Complutense University of Madrid, Spain

Abstract

GFI1B was identified by sequence homology with the oncogene GFI1 (Growth Factor Independence 1). Both GFI1 and GFI1B transcription factors have six C-terminal C2H2 zinc-fingers and an N-terminal SNAG (SNAIL/GFI1) transcriptional repression domain. While GFI1 is essential for neutrophil differentiation and is also necessary for B and T lymphopoiesis, GFI1B is required for development of erythroid and megakaryocytic lineages. However, mounting evidences indicate that GFI1B has also a prominent role in hematopoietic stem cell quiescence and, according to its function in cell differentiation and stem cell maintenance, in cancer. Here we will briefly review the last findings on these aspects.

Keywords: GFI1B; Stem cell; Hematopoiesis; Cancer; Leukemia; Lymphoma

GFI1 and GFI1B Role in Hematopoiesis

Hematopoiesis, the process of blood cells formation, has established the paradigm for cell differentiation from tissue specific stem cells, the Hematopoietic Stem Cells (HSC). This model is particularly relevant to understand the pathogenesis of blood malignancies. Hematopoiesis is regulated by transcription factors (TFs) that are essential to establish the normal cell balance and they also play a fundamental role in disease, particularly in cancer. Growth Factor Independence 1 (GFI1) and its homolog GFI1B are lineage-specific TFs required for hematopoiesis. GFI1B was identified by its sequence homology with GFI1 [1,2]. In fact, GFI1 and GFI1B have two domains with over 95% identity. The conserved N-terminal SNAG domain contains 20 amino acids, which recruit proteins that modify histones [2-4]. This domain has a nuclear localization motif and plays an important role in transcriptional repression through the binding of cofactors lysine-specific histone demethylase 1A (KDM1A, also known as LSD1) and RCOR1/2 (COREST) [3,5,6].

Both GFI1 and GFI1B important in the differentiation of the first adult type HSCs from common endothelial-blood progenitors in the aorta- gonad- mesonephros region, silencing the endothelial program. However, the expression pattern of both genes is different: GFI1 is specifically expressed within the dorsal aorta in endothelial cells and cells within emerging intra-aortic hematopoietic clusters, whereas GFI1B expression is more associated with the fully formed intra-aortic hematopoietic clusters [9]. Conditional knockout mice indicated Gfi1b is required for HSC quiescence. In these experiments, Gfi1b deficient mice show expansion of functional HSCs in the bone marrow and blood with high production of reactive oxygen species (ROS) (Figure 1) [10]. Further inactivation of Gfi1 suggested that both TFs can partly compensate each other, but at least one of them is required by HSCs. In agreement with that, Gfi1b is highly expressed in HSCs and its expression decreases with differentiation to Multi Potential Progenitors (MPPs). In contrast, Gfi1 shows lowest levels in HSCs and is up regulated in the MPP fractions (Figure 1) [10]. Cell fate modification experiments have further supported the importance of Gfi1b for HSC biology. In fact, Gfi1b is among the essential TFs sufficient to generate HSCs. Mouse embryonic fibroblasts can be transformed into endothelial-like cells that subsequently generate hematopoietic progenitor cells (HPCs) in vitro by over-expressing Gfi1b, c-Fos, Gata2 and Etv6. Although Etv6 increases the efficiency of this process, the first three TFs are enough to
achieve it [11]. More recently, Tsukada and colleagues have shown that teratomas derived from iPSCs obtained by reprogramming mice fibroblasts with Oct4, Sox2, and KIf4, generate in vivo functional long-term HSCs when hyper-express Gfi1b, c-Fos, and Gata2 [12]. GFI1 and GFI1B are also essential for lineage-specific differentiation. Knockout (KO) mice have revealed that Gfi1 is required for neutrophil differentiation; consistently, human GFI1 mutations are associated with severe congenital neutropenia [13,14]. Gfi1 is also necessary for B and T lymphopoiesis. Besides, Gfi1 is expressed in precursors of sensory neurons, the retina, specific lung cells, and in the central nervous system [15]. Instead, GFI1B is critical for expansion and differentiation of erythroid progenitors. Gfi1b KO embryos die by day E15 because of the lack of enucleated erythrocytes. Gfi1b KO mice also fail to develop megakaryocytes, but have arrested erythroid and megakaryocytic precursors in the fetal liver [16]. Loss of Gfi1b in adult mice stops erythroid development at an early progenitor stage, and blocks terminal megakaryocytic differentiation in the polyploid promegakaryocytes that fail to produce platelets [17]. In addition, moderate levels of GFI1B are expressed in immature B-cells, a subset of early T-cell precursors and peripheral blood granulocytes and monocytes [18,19]. GFI1B is very low or absent in Lymphoid-Primed Multipotent (LMPP), Common Lymphoid (CLP), Early Thymocyte (ETP), and Granulocyte-Monocyte Progenitors (GMPs) [20]. It seems that the short GFI1B form is relevant for erythroid development as well as showing a stronger repressor activity than its long counterpart [21]. The long GFI1B variant, for its part, has been found to be required for megakaryopoiesis, unlike the short variant which may, on the other hand, have an inhibitory effect on platelet production [22,23]. It has also been found that in vitro over-expression of Gfi1b inhibits myeloid differentiation of a myelomonocytic cell line, and that the lack of Gfi1 and Gfi1b expression produces a severe block in B cell development [2]. Although both proteins can greatly compensate for each other’s loss, they play unique differential roles in vivo. Consistent with this, Gfi1 hyper-expression can rescue erythroid and early megakaryocytic differentiation from adult mouse Gfi1b KO, but terminal megakaryocyte maturation defect cannot be compensated by Gfi1 or a Gfi1b hybrid containing the Gfi1 N-terminal portion [17]. These differences are patent in the inner ear where Gfi1b cannot replace Gfi1 function [24]. These findings show that GFI1B plays a major role in hematopoiesis. Its importance is also reflected by the strong control of its expression by several regulatory elements, particularly downstream the gene sequence, that bind both transcriptional activators and repressors [20,25-27]. This suggests that an appropriate GFI1B level may be relevant for its function.

**GFI1B and Cancer**

Consistent with Gfi1b function in megakaryopoiesis, different mutations in GFI1B are involved in platelet conditions [16,17,28]. However, in this review we shall focus on the recent insights on GFI1B role in cancer, particularly of hematopoietic lineages. When considering the development of acute leukemia, both mutations that block differentiation and those which promote proliferation or cell survival have been deemed necessary [29]. Besides its role in cell differentiation, GFI1B has pro-apoptotic activity when expressed in human CD34+ cells, disruption of these functions may contribute to leukemogenesis [30]. Initial expression studies found high levels of GFI1B in some primary CD34+ cells from human Acute Myeloid Leukemias (AMLs) and leukemic cell lines of erythroid and megakaryocytic lineages. Silencing GFI1B in these cell lines reduced proliferation and increased apoptosis [31]. High level of GFI1B expression was also observed in Chronic Myeloid Leukemia (CML), other Myelo Proliferative Neoplasms (MPN), AML, and B-lymphoblastic leukemias. Remarkably, the short GFI1B isoform was highly expressed in the leukemic cells. However, both isoforms were higher in CML after treating with tyrosine kinase inhibitors [32]. Silencing of both BCR-ABL1 and GFI1B in K562 CML cell line showed a cooperative anti-proliferative and pro-apoptotic effect [33]. In this context, the short form may be acting as a repressor over the long species. However, the low number of patients and controls analyzed implies that the conclusions of these works have to be taken with caution. In contrast, several pieces of evidence point to a GFI1B involvement in leukemia when its repressor function is reduced. In keeping with this, Gfi1b represses oncogene Meis1 [5]. Additionally, we have described a dominant negative GFI1B mutation, Asp262Asn
exclusive activation has been associated with medulloblastoma. In was decreased in the first lymphomas compared with the latest. transgene, but it is not in the control ones. Again, Gfi1b expression Gfi1b, but no Gfi1 (Figure 1). Gfi1b increased expression in these no normal unmutated individuals [38]. We also found GFI1B patients and normal carriers of JAK2 V617F mutation, but not with g.135870130C>G in GRCh37/hg19), has been associated with MPN A C>G transversion in GFI1B downstream region (rs621940, GFI1B regulatory elements may also take part in blood neoplasms. Interestingly, the short form has been described to interact more the short form when it predominates, is something to be elucidated. is only cell context dependent and if it is related to the inhibition of this, GFI1B has turned out to be an anti-leukemia target. A recent report shows that a LSD1 inhibitor disrupts the LSD1–GFI1B interaction, inducing de-repression of GFI1B target genes (particularly PU.1), causing granulomonocytic transdifferentiation [37]. This recapitulates to great extent the dominant negative GFI1B mutant effect (Figure 1). However, in GFI1B expressing acute erythroleukemia and acute megakaryoblastic leukemia cell lines the drug has anti-leukemic effect both in xenograft and in vitro models that could be attributed to the cell identity modification. If this action is only cell context dependent and if it is related to the inhibition of the short form when it predominates, is something to be elucidated. Normally, the short form has been described to interact more efficiently with LSD1 than the full-length one [21]. Changes in GFI1B regulatory elements may also take part in blood neoplasms. A C>G transversion in GFI1B downstream region (rs621940, g.135870130C>G in GRCh37/hg19), has been associated with MPN patients and normal carriers of JAK2 V617F mutation, but not with normal unmutated individuals [38]. We also found GFI1B promoter mutations in human leukemias. Nevertheless, no clear connection between these mutations and the disease was demonstrated [39]. Like GFI1, GFI1B has been associated to the pathogenesis of lymphoid malignancies. Consistent with the importance of GFI1B block in myeloid leukemias, TCF3 (E2A) prevention of T-lymphocyte progenitor transformation relies on GFI1B repression [40,41]. TCF3 is involved in human lymphoid leukemias and Tcf3 KO develops T-cell lymphoma. Ectopic expression of Tcf3 in Tcf3-/- cells induces growth arrest and apoptosis, together with direct up-regulation of Gfi1b, but no Gfi1 (Figure 1). Gfi1b increased expression in these cells has the same consequences [42]. Another finding supporting the implication of GFI1B reduction in lymphoma comes from those expressing BCL6 (B-Cell Lymphoma 6). This is relevant because this gene is frequently expressed in T and B cell lymphomas. Also, BCL6 chromosomal rearrangements and/or mutations are associated with human lymphomas [43]. Gfi1b is a retrovirus integration site in diffuse large B cell lymphomas of mice containing the human BCL6 transgene, but it is not in the control ones. Again, Gfi1b expression was decreased in the first lymphomas compared with the latest. Besides, GFI1B was decreased in human BCL6 positive T and B cell lymphomas [44]. Outside the blood setting, GFI1B or GFI1 mutually exclusive activation has been associated with medulloblastoma. In most cases this is due to structural changes that juxtapose the coding sequences to active enhancer elements [45]. Paradoxically, these observations suggest that GFI1B contributes to cancer development when its expression is increased or reduced. More data is required to understand the context effect or the splicing variant contribution. However, other genes have been related to malignancy both when up regulated and functionally inactivated, including key regulators of hematopoiesis like PU.1 which is, as we said, a GFI1B target [35]. This may be the case of other critical factors for stem cell biology regulation, commitment and differentiation [36,46,47].

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