Platelets

ISSN: 0953-7104 (Print) 1369-1635 (Online) Journal homepage: http://www.tandfonline.com/loi/iplt20

Differential expression of SDF-1 receptor CXCR4 in molecularly defined forms of inherited thrombocytopenias

Juan P. Salim, Ana C. Glembotsky, Paola R. Lev, Cecilia P. Marin Oyarzún, Nora P. Goette, Felisa C. Molinas, Rosana F. Marta & Paula G. Heller

To cite this article: Juan P. Salim, Ana C. Glembotsky, Paola R. Lev, Cecilia P. Marin Oyarzún, Nora P. Goette, Felisa C. Molinas, Rosana F. Marta & Paula G. Heller (2016): Differential expression of SDF-1 receptor CXCR4 in molecularly defined forms of inherited thrombocytopenias, Platelets, DOI: 10.1080/09537104.2016.1254763

To link to this article: http://dx.doi.org/10.1080/09537104.2016.1254763

Published online: 29 Dec 2016.

platelets

http://www.tandfonline.com/iplt
ISSN: 0953-7104 (print), 1369-1635 (electronic)

Platelets, Early Online: 1–5
 DOI: 10.1080/09537104.2016.1254763

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Differential expression of SDF-1 receptor CXCR4 in molecularly defined forms of inherited thrombocytopenias

Juan P. Salim, Ana C. Glembotsky, Paola R. Lev, Cecilia P. Marin Oyarzún, Nora P. Goette, Felisa C. Molinas, Rosana F. Marta, & Paula G. Heller

Hematology Research, Institute for Medical Research (IDIM) A. Lanari, University of Buenos Aires (UBA), National Council for Medical Research (CONICET), Buenos Aires, Argentina

Abstract

The SDF-1-CXCR4 axis plays an essential role in the regulation of platelet production, by directing megakaryocyte (MK) migration toward the vascular niche, thus allowing terminal maturation and proplatelet formation, and also regulates platelet function in an autocrine manner. Inherited thrombocytopenias (IT) comprise a spectrum of diverse clinical conditions caused by mutations in genes involved in platelet production and function. We assessed CXCR4 expression and SDF-1 levels in a panel of well-characterized forms of IT. Decreased surface CXCR4 levels were found in 8 of 27 (29.6%) IT patients by flow cytometry, including 4 of 6 patients with *ANKRD26*-RT, 3 of 3 patients with GPS and 1 of 6 patients with FPD/AML. Low CXCR4 levels were associated with impaired SDF-1-triggered platelet aggregation, indicating that this decrease is functionally relevant, whereas a normal platelet response was shown in patients harbouring preserved membrane CXCR4. Reduced CXCR4 was not due to decreased gene expression, as platelet RNA levels were normal or increased, suggesting a post-transcriptional defect. Increased ligand-induced receptor internalization was ruled out, as circulating SDF-1 levels were similar to controls. MK CXCR4 expression was normal, indicating that the defect in CXCR4 arises after the step of platelet biogenesis. In conclusion, the finding of defective CXCR4 expression specifically associated with certain IT disorders highlights the fact that abnormalities in several megakaryocytic regulators underlie IT pathogenesis and further reveal the heterogeneous nature of these conditions.

Keywords

CXCR4, inherited thrombocytopenias, SDF-1

History

Received 20 August 2016
Revised 5 October 2016
Accepted 21 October 2016
Published online 29 December 2016

Introduction

Megakaryocyte (MK) development initiates in the bone marrow endosteal niche, which promotes hematopoietic commitment and MK progenitor proliferation, while suppressing terminal maturation. Further migration to the sinusoidal vascular niche facilitates late steps of MK maturation and proplatelet protrusion through the endothelial barrier to release platelets into the bloodstream during thrombopoiesis. Previous work has revealed that the chemokine stromal cell-derived factor-1 (SDF-1) and its natural receptor, CXCR4, are main players implicated in MK chemotaxis toward sinusoidal vessels, driving MK location in the bone marrow to allow adequate platelet production [1–3]. Platelets are also sensitive to this chemokine, which is able to induce activation and release of platelet granules by itself and to potentiate the response to other agonists [4]. Moreover, platelets store and secrete SDF-1 upon activation, which turns this chemokine into an autocrine regulator of platelet function [5]. The participation of the SDF-1/CXCR4 axis in disease states involving the megakaryocytic lineage has been demonstrated by the finding of abnormal levels of CXCR4 in platelets and MKs from patients with essential thrombocythemia [6].

Inherited thrombocytopenias comprise a rare although increasingly recognized group of disorders characterized by diverse clinical presentation, genetic features and pathogenesis [7]. Due to the phenotypic heterogeneity and paucity of diagnostic biomarkers, it is often difficult to adequately characterize these patients. Abnormal platelet production underlies most forms of IT, and in many cases, it is associated with a platelet function defect that contributes to the bleeding tendency. Although to date more than 30 genes have been identified to cause ITs, the mechanisms by which mutations in these genes lead to thrombocytopenia and platelet dysfunction remain incompletely clarified [8]. In the present work, we studied CXCR4 expression and SDF-1 levels in a spectrum of well defined forms of inherited thrombocytopenias (IT), including *ANKRD26*-related thrombocytopenia (RT), familial platelet disorder with predisposition to acute leukemia (FPD/AML), X-linked thrombocytopenia (XLT) secondary to *WAS* mutation, Gray Platelet Syndrome (GPS), monoallelic Bernard-Soulier syndrome (mBSS) and *MYH9*-related disease (*MYH9*-RD).

Methods

Patients and samples

Twenty-seven patients with molecularly characterized forms of IT and 24 healthy individuals, matched by age and gender, were

Juan P. Salim and Ana C. Glembotsky contributed equally to this work.

Correspondence: Paula G. Heller, Hematology Research, Institute for Medical Research (IDIM) A Lanari, University of Buenos Aires (UBA), National Council for Medical Research (CONICET). Combatientes de Malvinas 3150. Buenos Aires 1427. Argentina. E-mail: paulaheller@hotmail.com

Table I. Features of patients with inherited thrombocytopenia.

Pedigree (No. pts.)	Age (years)	Platelet Count ($\times 10^9$/L)	Platelet Size (μm)	Gene	Mutation	Diagnosis
I (4), II (2)	31.5 (11–49)	125 (60–145)	2.9 (2.5–3.4)	*RUNX1*	p.T219Rfs*8†	FPD/AML
III (4), IV (2)	39.5 (17–74)	91 (68–147)	2.8 (2.4–3)	*ANKRD26*	c.-127A>G†; c.-125A>G	*ANKRD26*-RT
V (2)	27(23–31)	18 (10–25)	1.7 (1.5–1.9)	*WAS*	p.Q52H†	XLT
VI (2), VII (1)	65 (10–69)	54 (30–65)	4 (3.7–4.2)	*NBEAL2*	p.Y729*†; p.1280* and p.H2159Q††	Gray Platelet Syndrome
VIII (2)	47.5 (33–62)	96 (76–115)	3.2 (3.1–3.2)	*GP1BA*	p.Y294*†	Monoallelic BSS
IX-XIII (6)	22 (10–31)	36 (17–70)	4.9 (3.7–7.4)	*MYH9*	p.A95Asn;† p.R702H (2);† p.R702C (2)	*MYH9*-RD HD mutation
XIV-XV (2)	27 (30–34)	66 (42–90)	4.9 (4.9–5)	*MYH9*	pD1424N;† p.M847_E85†	*MYH9*-RD TD mutation

FPD/AML means familial platelet disorder with predisposition to acute myelogenous leukemia; *ANKRD26*-RT, *ANKRD26*-related thrombocytopenia; XLT, X-linked thrombocytopenia; BSS, Bernard-Soulier syndrome; *MYH9*-RD, *MYH9*-related disease; HD, head-domain; TD, tail-domain.
Reference values for mean platelet diameter, 2.3–3.1 μL.
† previously described [10].
†† submitted.

included in this study after informed consent. Patient features are described in Table I. Plasma samples, platelet-rich plasma and washed platelets were prepared as described [9–11]. Bone marrow biopsies were obtained by standard procedures, formalin-fixed and embedded in paraffin, and samples from subjects who underwent bone marrow biopsy for non-haematological diseases were studied as controls. The study was approved by the Institutional Review Board.

Platelet surface CXCR4 levels by flow cytometry

Platelets (1×10^6) were incubated with phycoerythrin (PE)-conjugated anti-CXCR4 antibody (Becton-Dickinson, San José, CA, USA) during 1 hour, fixed with 1% PFA and acquired on a FACScan flow cytometer (Becton-Dickinson). Anti-CD61-FITC was used to identify the platelet population, and an irrelevant antibody from the same IgG subclass was used as isotypic control. A control sample was assayed in parallel to each patient sample, all in duplicate. Data were expressed as relative fluorescence intensity (RFI), by calculating the ratio between mean fluorescence intensity of the CXCR4 antibody and the corresponding isotypic control. The reference range was established as the mean ± 2 standard deviation of 10 healthy individuals studied simultaneously.

Measurement of platelet size

Mean platelet diameter (MPD) was determined by measuring the largest diameter of 100 platelets in May-Grünwald-Giemsa-stained blood smears by using the VideoTesT-Master image analysis software (St. Petersburg, USSR).

Platelet aggregation studies

After centrifugation at 200 *g* for 10 min., upper two-third of platelet-rich plasma was obtained, adjusted to 3×10^8/mL, challenged with 100 nM SDF-1 (PeproTech Inc. Rocky Hill, NJ, USA), and aggregation was evaluated using a Lumi-aggregometer (Chrono-Log Corp., Havertown, PA, USA).

Real-time RT-PCR analysis of platelet CXCR4 transcripts

RNA was isolated from leukodepleted washed platelets after red cell lysis followed by cDNA synthesis, as described [11]. Real-time PCR was performed using IQTM SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) in an iCycler (Bio-Rad Life Science). Primer sequences for CXCR4 were as follows: forward 5- AGGGGATCAGTATATACACTT-3 and reverse 5-TGCCCACAATGCCAGTTAAG-3. Quantification was carried out by the $2^{-\Delta\Delta C}{}_T$ method using GAPDH expression as an internal normalization control. Each sample was assayed in triplicate.

Immunohistochemical CXCR4 assay

Bone marrow sections were prepared and stained as described [6]. Briefly, samples were deparaffinized, rehydrated, and after blocking endogenous peroxidase activity, sections were incubated with 2.5% blocking serum followed by anti-CXCR4 rabbit polyclonal antibody (Abcam, Cambridge, UK) overnight at 4°C. Negative controls were performed by omitting the primary antibody. The LSAB detection kit (Dako, CA, USA) was used for visualization, and slides were counterstained with haematoxylin. Images of all available MKs in each specimen were obtained, average cytoplasm optical density (OD), excluding the nucleus, was registered for each cell, and median OD was calculated using the VideoTesT-Master Morphology software.

Plasma SDF-1α and TPO measurement

Plasma SDF-1α and thrombopoietin (TPO) levels were assayed in duplicate using enzyme-linked immunosorbent assay technique (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's recommendations.

Statistical analysis

Data were tested for normality and analysed by Mann–Whitney test, for comparison between two groups and by Kruskal–Wallis with Dunn's post-test for comparison among multiple groups. Correlations were assessed using Spearman test, using GraphPad Prism 5 (La Jolla, CA, USA). Two-sided *P* values <0.05 were considered significant.

Results

Diverse CXCR4 expression pattern in different IT disorders

Platelet surface CXCR4 levels were found to be heterogeneous among patients with different forms of IT, as shown in Figure 1A. Overall, decreased platelet CXCR4 expression was found in 8 of 27 (29.6%) IT patients, including 4 of 6 patients with *ANKRD26*-RT, 1 of 6 patients with FPD/AML and 3 of 3 patients with GPS. In patients with GPS and in the patient with FPD/AML who harboured low CXCR4 levels, the decrease in CXCR4 was confirmed on a separate sample drawn several months apart. Interestingly, the defect in CXCR4 did not affect all members of the same family, illustrating the phenotypic diversity already described for these

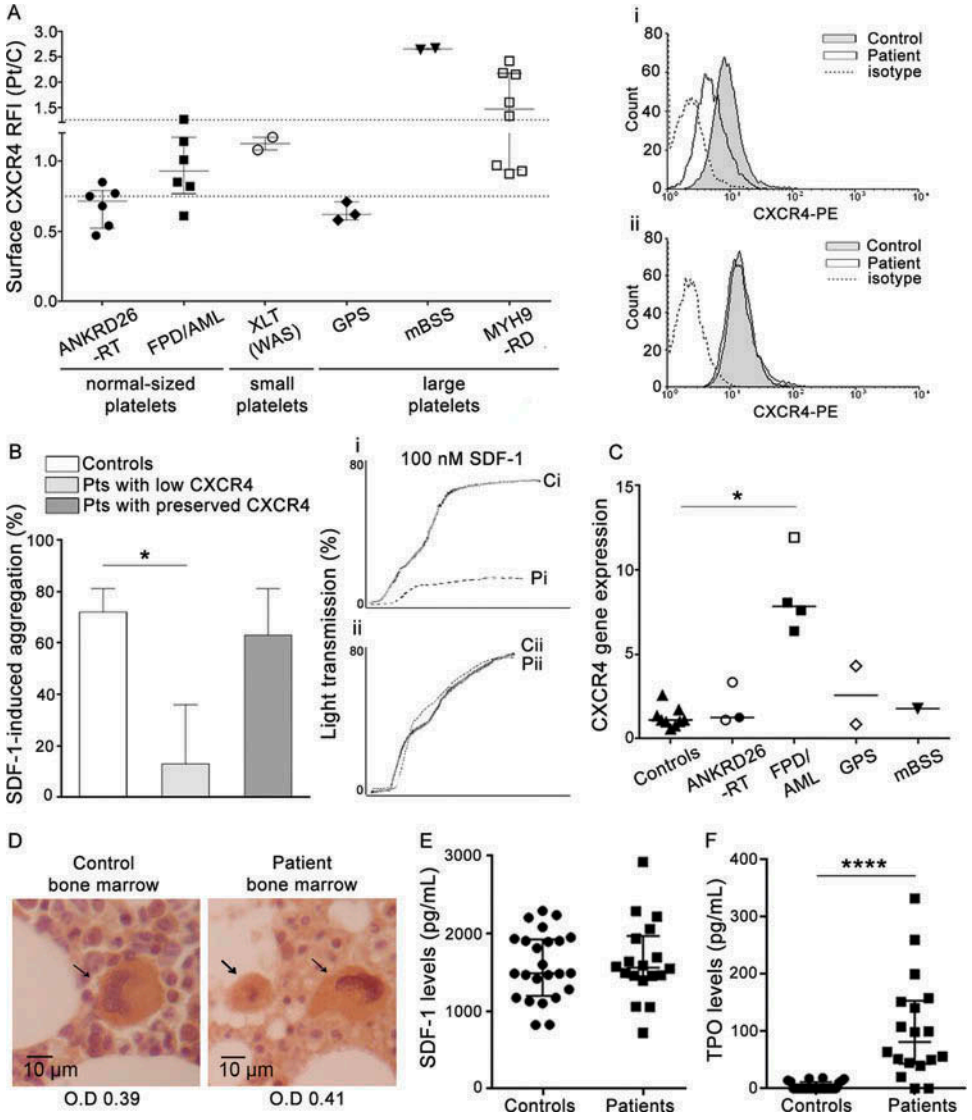

Figure 1. A. Surface CXCR4 expression by flow cytometry in patients with inherited thrombocytopenia classified as those harbouring normal-sized, small and large platelets according to measurement of the mean platelet diameter on blood smears, including *ANKRD26*-RT (n = 6), FPD/AML (n = 6), XLT (n = 2), GPS (n = 3), mBSS (n = 2) and *MYH9*-RD (n = 8). Results are expressed as the ratio between relative fluorescence intensity (RFI) of patient platelets and a control sample assayed in parallel (Pt/C). Median values with interquartile range are indicated for each group. Horizontal dashed lines represent upper and lower limits of the reference range, established by the mean ± 2 SD of controls. The right panel shows representative examples of a patient with low (i) and a patient with preserved (ii) CXCR4 surface expression, as depicted by the open black histograms while filled grey histograms show binding to simultaneously assayed control platelets. B. Platelet aggregation in response to 100 nM SDF-1 in patients (Pts.) with low surface CXCR4 levels (n = 3), patients with preserved CXCR4 levels (n = 3) and control subjects (n = 7). Bars represent median values with interquartile range. **P*<0.05, Kruskal–Wallis followed by Dunn´s multiple comparison test. Representative aggregation traces in (i) a patient with low CXCR4 levels (Pi) harbouring impaired SDF-1-triggered platelet aggregation) and (ii) an aggregation trace in a patient with preserved CXCR4 levels (Pii) showing normal platelet response compared to simultaneously assayed controls (Ci and Cii, respectively). C. CXCR4 gene expression in platelets. CXCR4 mRNA levels were assessed by real-time PCR in patients with *ANKRD26*-RT (n = 3), FPD/AML (n = 4), GPS (n = 2), mBSS (n = 1) and control subjects (n = 9). Open symbols in each data set indicate patients with low surface CXCR4. Horizontal lines represent median values. **P* < 0.05, Kruskal–Wallis followed by Dunn´s multiple comparison test. D. Megakaryocyte CXCR4 expression by immunohistochemistry in a FPD/AML patient and a control bone marrow sample. Mean optical density is depicted for each case. Arrows indicate megakaryocytes. Original magnification 40×. Scale bar 10 µm. Circulating SDF-1 (E) and TPO (F) levels in IT patients (n = 18) and controls (n = 24) measured by ELISA. Median values and interquartile range are depicted. ****P < 0.0001, Mann–Whitney test.

disorders [11,12]. The decrease in CXCR4 in the patient with FPD/AML was not related to myelodysplastic syndrome or leukemia, and this patient did not develop disease transformation after a 9-year follow-up. In contrast, CXCR4 expression was increased in certain forms of IT harbouring large platelets, including 2 patients with mBSS and 5 of 8 patients with *MYH9*-RD, probably related to increased platelet surface. Accordingly, platelet CD61 expression was, as expected, increased in this group of patients, patient/control RFI ratio, 2.15 ± 1.1, and correlation was found between CD61 and CXCR4 levels, r=0.68, P<0.05 (Spearman correlation test). Besides, despite decreased platelet size, CXCR4 levels were normal in patients with XLT. No correlation was found between CXCR4 levels and platelet count or mean platelet diameter in the whole patient population (data not shown), highlighting that the defect in CXCR4 expression is not a

reflection of altered platelet size and may be found in patients with normal-sized or even large platelets, as shown for GPS patients.

The pattern of SDF-1-induced platelet aggregation is related to CXCR4 expression

Platelet aggregation in response to SDF-1 was decreased in two patients with *ANKRD26-RT* and one patient with FPD/AML, all of whom had low CXCR4 expression, suggesting that the decrease in CXCR4 is of functional significance. In contrast, normal SDF-1-induced platelet aggregation was shown for 3 patients with large platelets, one with *MYH9*-RD and two with mBSS, all of whom had preserved CXCR4 levels (Figure 1B).

Low CXCR4 levels are not due to decreased gene expression

In order to investigate the mechanisms underlying decreased CXCR4, we next measured platelet CXCR4 gene expression. RNA levels were normal or increased in the different IT disorders (Figure 1C), including patients with low (n=5) and those with preserved (n=5) surface CXCR4, indicating that low CXCR4 levels on the platelet membrane are not due to decreased gene expression. Interestingly, CXCR4 mRNA levels were significantly higher in FPD/AML platelets than in controls, as shown in Figure 1C, but the mechanism leading to this finding and its potential relationship to *RUNX1* mutation remain unclear.

CXCR4 expression is preserved in bone marrow megakaryocytes

To assess whether low platelet CXCR4 reflects a decrease in MK CXCR4 levels or represents a peripheral abnormality, we evaluated its expression in available bone marrow samples from one with FPD/AML who had decreased CXCR4, an affected FPD/AML family member who had, in contrast, normal CXCR4 platelet levels, and a patient with MYH9-RD, who had preserved platelet CXCR4. Megakaryocyte CXCR4 levels in patients (n = 3) did not differ from controls (n = 3), 0.39 (0.39–0.47) vs. 0.40 (0.36–0.41), respectively, P = NS (Figure 1D), indicating that the reduction in CXCR4 is acquired during circulation.

Normal circulating SDF-1 levels in IT patients

Circulating SDF-1 in patients with IT (n = 18) did not differ from controls (n = 24), 1561 (722–2920) vs. 1488 (822–2292) pg/mL, respectively, P = NS (Figure 1E), without significant differences among different IT disorders. No correlation was found between SDF-1 levels and platelet counts, nor CXCR4 expression. In contrast, plasma TPO levels were significantly elevated in the IT group, 81.2 (0–333) vs. 0 (0–19) pg/mL, respectively, $P < 0.0001$, Mann–Whitney test (Figure 1F), as previously reported [9].

Discussion

The mechanisms underlying impaired platelet biogenesis in IT syndromes are diverse and remain incompletely understood. The defect may involve different stages of megakaryopoiesis, proplatelet formation or even affect preplatelet fission after proplatelet release to the circulation [7]. Mutations targeting transcription factors (RUNX1, GATA-1, FLI-1, ETV6, GFI1B, HOX-1, MECOM), their transcriptional targets (ANKRD26, RBM8A), cytoskeleton proteins (MYH9, ACTN1, WAS, FLNA), granule biogenesis (NBEAL2) and receptors for bone marrow extracellular matrix proteins (GPIb-IX, GPIIbIIIa) underlie some of the characterized forms, but in many cases, the molecular and cellular basis of IT is still unknown. Abnormal expression of several receptors for cytokines or classical agonists has been described in IT MKs and/or platelets, including Mpl [9], GPIa [12], GPVI [13] and PAR-1 [14]. These defects contribute to IT pathogenesis and may, in addition, represent potential diagnostic biomarkers.

Although the SDF-1 /CXCR4 axis is essential in regulating platelet production and function, its potential role in IT has remained poorly explored. In this study, we demonstrate defective expression of a main chemokine receptor, CXCR4, in IT patients, selectively associated with certain clinical conditions, including *ANKRD26*-RT, GPS and FPD/AML. In contrast, high CXCR4 levels have been previously reported in platelets from patients with immune thrombocytopenia [15]. In the current work, low CXCR4 levels in IT were restricted to platelets, as MK CXCR4 expression was found to be normal, indicating that the reduction in CXCR4 does not originate at the stage of platelet biogenesis but is acquired in circulation after platelet release. A similar scenario has been described for the reduction in GPIa levels shown in *ANKRD26*-RT, which is evident in platelets, but not in MKs [16]. Low platelet surface CXCR4 was not paralleled by a reduction in mRNA levels, indicating that this defect is not due to decreased gene expression but likely represents a post-transcriptional abnormality. Further study is required to determine the mechanisms underlying the decrease in CXCR4 in IT and its relationship to specific gene defects. Potential explanations could include abnormal intracellular trafficking, proteasomal degradation and receptor internalization [17]. In this regard, as opposed to TPO, circulating SDF-1 levels, which are able to induce down-regulation of CXCR4 through internalization [18], were not elevated in this patient cohort, rendering the latter possibility unlikely. Interestingly, we have previously shown a concomitant reduction in Mpl expression [9] in patients found to harbour low CXCR4 levels in the current study, including FPD/AML (n=1), ANKRD26-RT (n = 2) and GPS (n = 2). FPD/AML and *ANKRD26*-RT share several common features, including normal platelet size and predisposition to leukemia. Although a severe reduction in α-granules is a unique characteristic of GPS patients, those with *ANKRD26*-RT and FPD/AML also harbour a moderate reduction in α-granule content [11,12]. Whether the decrease in α-granules plays a role in the reduction in surface CXCR4 found in these three disorders remains speculative.

Besides regulating megakaryopoiesis, SDF-1 regulates the function of circulating platelets and platelet-derived SDF-1 exerts an autocrine/paracrine loop to enhance platelet aggregation, in a similar manner to ADP and TxA2. Pharmacologic or antibody-induced inhibition of CXCR4 signalling abrogates collagen-mediated platelet aggregation and dense granule release and inhibits thrombus formation in vivo [5]. In this study, low CXCR4 levels were associated with impaired SDF-1-induced platelet aggregation, suggesting that this abnormality is functionally relevant and could potentially contribute to impaired platelet function found in certain IT patients.

In conclusion, this study adds CXCR4 to the list of cellular abnormalities displayed by IT patients, highlighting that multiple defects in megakaryocytic regulators underlie IT pathogenesis. Further insight into the relationship between decreased CXCR4 and the underlying genetic defects may contribute to unravel the molecular pathways involved in the megakaryocytic lineage. The diversity in CXCR4 expression found among different IT conditions, and even among patients belonging to the same pedigree, further reveals the heterogeneous nature of these complex yet fascinating disorders.

Acknowledgments

We are grateful to Alessandro Pecci and Anna Savoia for their invaluable help with molecular analysis of IT patients.

Declaration of Interest statement

The authors report no declarations of interest. This work was supported by research grants from the National Agency for Scientific and Technological Research Promotion (ANPCyT), PICT 12-0629, and the National Council for Medical Research, PIP 11220120100489.

References

1. Hamada T, Möhle, R, Hesselgesser J, Hoxie J, Nachman RL, Moore MA, Rafii S. Transendothelial migration of megakaryocytes in response to stromal cell-derived factor 1 (SDF-1) enhances platelet formation. J Exp Med 1998;188:539–548.
2. Avecilla ST, Hattori K, Heissig B, Tejada R, Liao F, Shido K, Jin DK, Dias S, Zhang F, Hartman TE, et al. Chemokine-mediated interaction of hematopoietic progenitors with the bone marrow vascular niche is required for thrombopoiesis. Nat Med 2004;10:64–71.
3. Niswander LM, Fegan KH, Kingsley PD, Mc Grath KE, Palis J. SDF-1 dynamically mediates megakaryocyte niche occupancy and thrombopoiesis at steady state and following radiation injury. Blood 2014;124:277–286.
4. Massberg S, Konrad I, Schürzinger K, Lorenz M, Schneider S, Zohlnhoefer D, Hoppe K, Schiemann M, Kennerknecht E, Sauer S, et al. Platelets secrete stromal cell–derived factor 1α and recruit bone marrow–derived progenitor cells to arterial thrombi in vivo. J Exp Med 2006;203:1221–1233.
5. Walsh TG, Harper MT, Poole AW. SDF-1α is a novel autocrine activator of platelets operating through its receptor CXCR4. Cell Signal 2015;27:37–46.
6. Salim JP, Goette NP, Lev PR, Chazarreta CD, Heller PG, Alvarez C, Molinas FC, Marta RF. Dysregulation of SDF-1/CXCR4 axis in the megakaryocytic lineage in Essential Thrombocythemia. Br J Haematol 2009;144:69–77.
7. Balduini CL, Melazzini F, Pecci A. Inherited thrombocytopenias-recent advances in clinical and molecular aspects. Platelets 2016 May 9:1–11. DOI:10.3109/09537104.2016.1171835.
8. Lentaigne C, Freson K, Laffan MA, Turro E, Ouwehand WH; BRIDGE-BPD consortium and the thrombogenomics consortium. Inherited platelet disorders: toward DNA-based diagnosis. Blood 2016;127:2814–2823.
9. Heller PG, Glembotsky AC, Goette NP, Marta RF, Lev PR, Molinas FC. Diverse Mpl expression pattern among pedigrees with inherited thrombocytopenia: potential diagnostic and therapeutic implications. J Thromb Haemost 2008;6:2215–2217.
10. Glembotsky AC, Marta RF, Pecci A, De Rocco D, Gnan C, Espasandin YR, Goette NP, Negro F, Noris P, Savoia A, et al. International collaboration as a tool for diagnosis of patients with inherited thrombocytopenia in the setting of a developing country. J Thromb Haemost 2012;10:1653–1661.
11. Glembotsky AC, Bluteau D, Espasandin YR, Goette NP, Marta RF, Marin Oyarzun CP, Korin L, Lev PR, Laguens R, Molinas FC, et al. Mechanisms underlying platelet function defect in a pedigree with FPD/AML: potential role for candidate RUNX1-targets. J Thromb Haemost 2014;12:761–772.
12. Noris P, Perrotta S, Seri M, Pecci A, Gnan C, Loffredo G, Pujol-Moix N, Zecca M, Scognamiglio F, De Rocco D, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. Blood 2011;117:6673–6680.
13. Nurden AT, Nurden P. The gray platelet syndrome: clinical spectrum of the disease. Blood Rev. 2007;21:21–36.
14. Larocca LM, Heller PG, Podda G, Pujol-Moix N, Glembotsky AC, Pecci A, Alberelli MA, Balduini CL, Landolfi R, Cattaneo M, et al. Megakaryocytic emperipolesis and platelet function abnormalities in five patients with gray platelet syndrome. Platelets 2015;26:751–757.
15. Wang JD, Ou TT, Wang CJ, Chang TK, Lee HJ. Platelet apoptosis resistance and increased CXCR4 expression in pediatric immune thrombocytopenic purpura. Thromb Res 2010;126:311–318.
16. Bluteau D, Balduini A, Balayn N, Currao M, Nurden P, Deswarte C, Leverger G, Noris P, Perrotta S, Solary E, et al. Thrombocytopenia-associated mutations in the ANKRD26 regulatory region induce MAPK hyperactivation. J Clin Invest 2014;124:580–591.
17. Marchese A. Endocytic trafficking of chemokine receptors. Curr Opin Cell Biol 2014;27:72–77.
18. Chatterjee M, Seizer P, Borst O, Schönberger T, Mack A, Geisler T, Langer HF, May AE, Vogel S, Lang F, Gawaz M. SDF-1α induces differential trafficking of CXCR4-CXCR7 involving cyclophilin A, CXCR7 ubiquitination and promotes platelet survival. FASEB J 2014;28:2864–2878.