Current Concepts on the Pathogenesis of the Hypereosinophilic Syndrome/Chronic Eosinophilic Leukemia

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Abstract: Chronic eosinophilic leukemia is a clonal disease characterized by hypereosinophilia and eosinophilia-related pathologic manifestations. Recently, the fusion gene *FIP1L1/PDGFRA* was found in the long arm of chromosome 4 and its expression has been shown to be associated with development of a clinical hypereosinophilic syndrome (HES) in a significant proportion of patients. FIP1L1/PDGFR*α*, the product of the gene *FIP1L1/PDGFRA*, is a constitutively activated tyrosine kinase and can be inhibited by imatinib mesylate. Several investigations have tried to dissect the mechanism of leukemogenesis and signaling induced by FIP1L1/PDGFR*α* in cell lines, primary human eosinophils and in murine myeloproliferative models. In this review, we analyzed the current knowledge on the relationship between FIP1L1/PDGFR*α*-induced signaling and eosinophil proliferation, survival and activation, specially focusing on its possible role in the modulation of cytokine and chemoattractant signaling pathways.

Keywords: FIP1L1/PDGFR*α*, chronic eosinophilic leukemia, hypereosinophilic syndrome eosinophils, mast cells.

Introduction

Hypereosinophilic syndrome (HES) was first described by Hardy and Anderson in 1968 (Hardy and Anderson, 1968). The diagnostic criteria of this hematological disorder were proposed by Chusid et al. in 1975 (Chusid et al. 1975) and have been almost invariably accepted until recently. These criteria include unexplained severe peripheral blood eosinophilia (higher than 1500 eosinophils/mm^3) sustained for over 6 months and accompanied by end-organ damage resulting from direct organ infiltration by eosinophils. The generic term "unexplained eosinophilia" has been used to exclude any allergic, inflammatory, infectious or neoplastic diseases, including specific eosinophilia induced by chronic or acute myelogenous leukemia, myelodysplastic syndromes or other myeloproliferative disorders.

In 2001, the World Health Organization (WHO) proposed a set of criteria that distinguish chronic eosinophilic leukemia (CEL) from HES (Bain et al. 2001). These criteria were based on the exclusion of the diseases mentioned above, together with the absence of a T-cell population with an aberrant phenotype and abnormal cytokine production, and the presence of a clonal cytogenetic abnormality or clonality, or a blast content in the peripheral blood (higher than 2%) or marrow (more than 5% but less than 19%). However, there was still the general consensus to use HES as a broad category to define heterogeneous conditions having hypereosinophilia and eosinophilic tissue infiltrations (Klion et al. 2006). Two types of HES/CEL, in particular have been described; Intrinsic: those with clonal expansion of a myeloid progenitor population with primary eosinophil differentiation including FIP1L1/PDGFRα^+ CEL and CEL demonstrating with cytogenetic abnormalities; and Extrinsic: eosinophil expansion responding to a clonal expansion of T-cells, expressing high levels of the eosinophil-differentiating cytokine interleukin-5 (IL-5), including a subgroup of HES/CEL patients who present clonal T-cell populations, expressing aberrant phenotypes and producing Th2 cytokines such as IL-5 (Bank et al. 2001, Cogan et al. 1994, Raghavachar et al. 1987; Simon et al. 1999).

To examine the role of genetic abnormalities in cases of hypereosinophilia, multiple methods have been used to diagnose clonal disorders, including classical cytogenetical analysis of purified eosinophils, fluorescent in situ hybridization, cytogenetic analysis of purified eosinophils and X-chromosome inactivation analysis through the human androgen receptor gene analysis (HUMARA) (Chang et al. 1999). However, the analysis of clonality in HES may have been limited in these studies by a low frequency of chromosomal anomalies and a dramatic male predominance (Gilliland et al. 2004;

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Pardanani et al. 2006) that prevented X-inactivation studies. More recent analysis of other cohorts has not shown this male predominance (M.E. Rothenberg, personal communication). The chromosomal abnormalities include trisomy 8 (Guitard et al. 1994; Ma et al. 1995; Quiquandon et al. 1995; Weinfeld et al. 1977), deletion of chromosome Y (Needleman et al. 1990); t(8;9)(p21–23;p23–24)(Reiter et al. 2005); del(6)(q24) and ins(9;4) (q34;q12q31) (Schoch et al. 2004). Interestingly, many of the cases of clonal eosinophilia are associated with lymphoproliferative disorders (T-cell lymphomas, Hodgkin's lymphoma or acute lymphoblastic leukemia), mastocytosis or other chronic myeloproliferative disorders. Also, it includes some specific subtypes of acute myeloid leukemia affecting the chromosome 16 (M4Eo inv(16)(p13q22) or t(16;16)(p13;q22)) (Le Beau et al. 1985; Marlton et al. 1995), resulting in chimeric fusion of the CBFb and MYH11 genes; t(5;16)(q33;q22) (Bhambhani et al. 1986), and t(16;21)(p11;q22) (Mecucci et al. 1985); the leukemia M2 with t(8;21)(q22;q22) (Swirsky et al. 1984) which links the acute myeloid leukemia-1 (AML1) and eight-twenty-one (ETO) genes; the presence of monosomy 7 (Song and Park 1987) or trisomy 1 (Harrington et al. 1988); t(10;11)(p14;q21) (Broustet et al. 1986), and rare eosinophil myelodysplastic syndromes (t(1;7) or dic(1;7)) (Matsushima et al. 1995).

These findings only accounted for a small fraction of patients with HES. In fact, until recently, the absence of significant specific markers of clonality made the diagnosis of CEL to be based on indirect clinical or laboratory data, such as the presence of hepatosplenomegaly, morphological dysplasia of eosinophils or other cell lineages, myeloid immaturity, bone marrow (BM) fibrosis, elevated serum vitamin B12 or mastocytosis-associated elevated serum tryptase levels (Roufosse et al. 2004). Since chronic myelogenous leukemia (CML) shares many of these clinicobiological features, there was often confusion about when to apply the diagnosis of CEL. Therefore, most of patients with the criteria defined by Chusid et al. remained diagnosed as HES.

The finding that a novel fusion gene, called FIP1 like 1/platelet derived growth factor receptor alpha *(FIP1L1/PDGFRA)* is responsible for a significant proportion of HES/CEL cases has changed the expectations of diagnosis and therapy in this disease, and has modified the view about incidence of clonal disorders in HES (Cools et al. 2003a).

In this review, we focus on the new advances in the pathogenesis and molecular mechanisms involved in CEL and the development of animal models that can explain the pathogenesis, clinical presentation and therapeutic targets for FIP1L1/PDGFRα-induced CEL.

Pathogenesis of FIP1L1/PDGFRα-induced HES/CEL

In 2001, a group reported a successful response to administration of imatinib mesylate in one patient with HES, that had been unsuccessfully treated with interferon-alpha and hydroxyurea (Schaller and Burkland, 2001). The rationale of its use was based on the efficacy demonstrated of imatinib in another myeloproliferative disease, BCR (breakpoint cluster region)/ABL (Abelson leukemia virus gene)-positive CML (Deininger and Druker, 2003; Druker et al. 1996). Imatinib was used and led to a rapid remission without any significant side effects. Subsequently, several groups reported more HES individuals who responded to imatinib (Ault et al. 2002; Cortes et al. 2003; Gleich et al. 2002; Pardanani et al. 2003b). When these studies were combined, 40–80% patients showed complete remission after imatinib therapy (Cortes et al. 2003; Gleich et al. 2002; Pardanani et al. 2003b). However, the molecular basis of the imatinib response remained unclear. In 2003, two independent groups used different approaches to identify the molecular mechanism of imatinib responsiveness. The first group found an imatinib-responder patient who presented the translocation t(1;4)(q44;q12) (Cools et al. 2003a). The combination of this patient's response to imatinib (able to inhibit not only BCR/ABL-induced proliferation but also c-kit and platelet-derived-growth factor receptor beta), and the finding of a translocation at the chromosomal region 4q12, which contained the genes *PDGFRA* and *cKIT*, led to the discovery of a chimeric *PDGFRA* transcript with the novel gene *FIP1L1* (from chromosomal region 4q12 and homologue to the *FIP1* gene from *Saccharomyces cerevisiae*) translating the FIP1L1/PDGFRα fusion protein. A second group identified an eosinophil cell line (Eol-1) which proliferation was inhibited by imatinib. The cell line expressed a novel 110 kDa phosphorylated fusion protein, composed of an N-terminal region, encoded by a gene of unknown function (that corresponded to the same gene that had just been named *FIP1L1*), and the

C-terminal region derived from the intracellular domain of PDGFRα (Griffin et al. 2003).

That a tyrosine kinase domain is responsible to develop CEL has been emphasized by the finding of a significant number of cases of myeloproliferative disorders with eosinophilia, where there were other fusion genes affecting the *PDGFRA* gene, or other genes that coded for tyrosine kinase receptors like *PDGFRB* and *fibroblast growth factor receptor 1 (FGFR1)*. An excellent review of these fusion genes and others that induce eosinophilia or hypereosinophilia, usually associated with neutrophilic myeloproliferation has been recently published (Tefferi et al. 2006).

The *FIP1L1/PDGFRA* fusion gene is generated by an approximately 800 Kb interstitial chromosomal deletion on 4q12 (Figure 1). Sequencing of the resulting *FIP1L1/PDGFRA* fusion gene revealed breakpoints scattered in FIP1L1, between exons 7 and 13 that affected coding and non-coding sequences, whereas breakpoints in *PDGFRA* gene are exclusively restricted to exon 12 of chromosome 4, affecting the coding sequence of its juxtamembrane (JM) region, which is known to have an autoinhibitory function (Cools et al. 2003a; Roche-Lestienne et al. 2005; Vandenberghe et al. 2004). The translated protein also includes the full tyrosine kinase domain which becomes constitutively activated (see below).

For detection of the *FIP1L1/PDGFRA* fusion gene, FISH analysis and/or RT-PCR are necessary. The interstitial chromosomal deletion on 4q12 of FIP1L1/PDGFRα^+ patients includes the cysteine-rich hydrophobic domain 2 (*CHIC2*) locus. Nested or real-time RT-PCR of the *FIP1L1/PDGFRA* mRNA, on purified peripheral blood leukocytes or purified eosinophils, and analysis of the loss of *CHIC2* locus on 4q12 by FISH analysis as a surrogate marker (Pardanani et al. 2003a) (Fig. 1), have been used to diagnose the presence of such an interstitial deletion. Expression of the *FIP1L1/PDGFRA* fusion gene or deletion of the surrogate marker CHIC2 have been detected in non-eosinophilic cells, including neutrophils, monocytes, mast cells, BM CD34$^+$ cells and even lymphoid cells from a fraction of patients, suggesting that the fusion of the *FIP1L1* and *PDGFRA* genes may occur in hematopoietic stem cells or early progenitors (HSC/P) (Pardanani et al; 2003a, Robyn et al. 2006; Tefferi et al. 2004).

Overall, the incidence of FIP1L1/PDGFRα^+ HES/CEL patients ranged from 4% to 60% (Table 1), becoming the most frequent clonal defect demonstrated in HES/CEL. The expression of the *FIP1L1/PDGFRA* gene especially correlated with a severe subtype of HES, called myeloproliferative variant. The wide range in the frequency of expression of the *FIP1L1/PDGFRA* gene may derive from the use of different diagnostic techniques, as well as biased patient selection. Notably, a relatively-frequent subgroup of FIP1L1/PDGFRα^+ patients also have elevated serum tryptase levels, BM infiltration of mast cells (which presented an abnormal spindle shape), and/or expression of the low-affinity IL-2 receptor (CD25). These are parameters associated with systemic mastocytosis (Klion et al. 2003; Pardanani et al. 2003a; Tefferi et al. 2004). While a physiological relationship between mast cells and

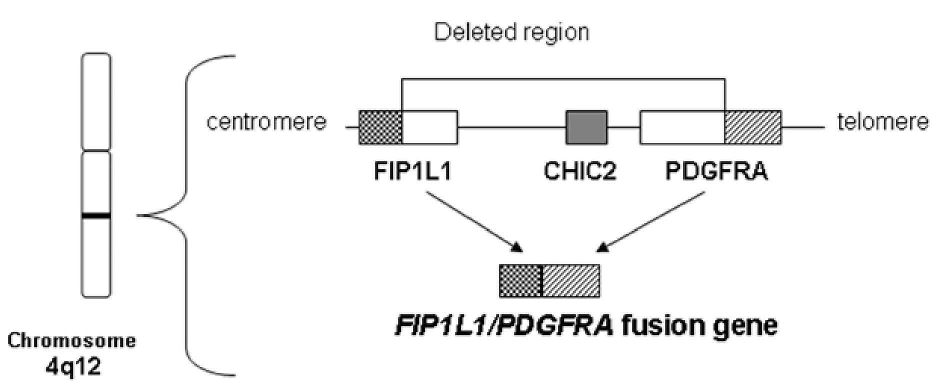

Figure 1. Interstitial deletion of 4q12 resulting in generating *FIP1L1/PDGFRA* fusion gene.
The FIP1L1/PDGFRA fusion gene is generated from the fusion of FIP1L1 gene and PDGFRA gene by approximately 800kbp deletion(Cools et al. 2003a). This chromosomal deletion includes the cysteine-rich hydrophobic domain 2 (*CHIC2*) locus. Deletion of the *CHIC2* locus at 4q12 in fluorescence in situ hybridization (FISH) is a surrogate marker for FIP1L1/PDGFRA fusion gene(Pardanani et al. 2003a).

Table 1. Reports of patients with FIP1L1/PDGFRA fusion gene in primary hypereosinophilia patients

Year	Diagnosis	% F/P+ #	Diagnosis of F/P+ patients (No.)	Comments	references
2003	HES, AML-EoMPD	56 (9/16)	CEL (8), AML-Eos (1)**	A patient with F/P T674I mutation relapsed	(Cools et al. 2003a)
2003	HES	56 (5/9)	CEL (5)	Elevated serum tryptase (5/5)	(Klion et al. 2003)
2003	SM+Eos	60 (3/5)	SM+Eos (3)	D816V mutation (2/5)	(Pardanani et al. 2003a)
2004	M-HES	100 (7/7)	CEL (7)	Serum IL-5 levels were low or undetectable	(Klion et al. 2004b)
2004	HES/CEL	47 (8/17)	CEL (8)	Splenomegaly (5/8) Elevated serum vitamin B12 (8/8)	(Vandenberghe; et al. 2004).
2004	HES**	50 (2/4)	CEL (2)	Serum IL-5 levels were low	(Klion et al. 2004a)
2004	HES, c-Eos SM-Eos	14 (11/81)	CEL (1), SM+Eos (10)	Imatinib partial responder in non-F/P(4/17)	(Pardanani et al. 2004)
2004	HES	67 (2/3)	CEL (2)	Both patients had pruritus and pulmonary infiltrates	(Smith et al. 2004)
2005	HES	17 (6/35)	CEL (6)	TCRg rearrangement (11/35), Elevated tryptase of F/P+ (3/3)	(Roche-Lestienne et al. 2005)
2005	HES, un-Eos	38 (10/26)	CEL (10)	Significantly more frequent hepatosplenomegaly in F/P+ patients	(La Starza et al. 2005)
2006	un-Eos	10 (4/40)	CEL (3), AML-CEL (1)**	4/40 clonal eosinophilia including ins(9;4)(q34;q12q31), AML following CEL	(Bacher et al. 2006)
2006	HES/CEL	25 (2/8)	CEL (2)	MHES (1), F/P+CEL (2) and Ly-HES (1)	(Helbig et al. 2006)
2006	HES, un-Eos	11 (31/270)	CEL (31)	9/217 in non-F/P including KIF5B-PDGFRA fusion had PDGFRA overexpression	(Score et al. 2006)
2006	HES	4 (32/830)	CEL (32)	Update on previous report on 2004.	(Pardanani et al. 2006)

HES, hypereosinophilic syndrome; AML-EoMPD, acute myeloid leukemia following eosinophilic myeloproliferative disorder; SM-Eos, systemic mastocytosis with persistent eosinophilia; M-HES, myeloproliferative variant of HES; CEL, chronic eosinophilic leukemia; c-Eos,clonal eosinophilia; un-Eos, persistent unexplained eosinophilia, F/P, FIP1L1/PDGFRA fusion gene

* patients with HES refractory to or intolerant of therapy with corticosteroids, hydroxyurea, and interferon-α

** both AML patients following persistant hypereosinophilia had trisomy 8.

Between brackets, numerator: number of patients with FIP1L1/PDGFRα expression; denominator: number of patients with primary eosinophilia.

eosinophils is well documented (e.g. mast cells produce eosinophil-active cytokines and mediators including IL-5, GM-CSF, histamine, and leukotriene LTD_4, and eosinophils stimulate mast cells with their products such as major basic protein), there is a strong possibility that FIP1L1/PDGFRα directly affects both eosinophil and mast cell proliferation and function, or induces closer crosstalk between them. Consistent with a direct effect of FIP1L1/PDGFRα on mast cells, mast cells have been shown to be FIP1L1/PDGFRα^+ in these patients (Robyn et al. 2006).

Molecular mechanism of FIP1L1/PDGFRα activation and signaling

The FIP1L1/PDGFRα fusion protein is a dysregulated tyrosine kinase (Cools et al. 2003a). The physiological and pathological role of one of the fused genes, FIP1L1, remains unknown. A recent report has demonstrated that the activation of FIP1L1/PDGFRα tyrosine kinase domain depends on the integrity of the JM domain kinase of PDGFRα while the FIP1L1 is dispensable for FIP1L1/PDGFRα tyrosine kinase activation (Stover et al. 2006). The JM domain has been previously shown to play a crucial role in the autoregulation of other receptor tyrosine kinases (RTKs) (Hubbard, 2004). When the JM domain is disrupted by a mutation, the PDGFR tyrosine kinase becomes constitutively activated. Two conserved tryptophan residues in the JM domain are required for its normal inhibitory activity (Stover et al. 2006). Indeed, all the reported breakpoints of the *PDGFRA* gene in HES/CEL patients induce truncations of the JM-containing exon 12 (Cools et al. 2003a; Roche-Lestienne et al. 2005; Vandenberghe et al. 2004). Interestingly, similar to what has been described in BCR/ABL-induced CML (Li et al. 2001), an imatinib-resistant mutation in the *FIP1L1/PDGFRA* fusion gene has been identified that results in relapse after an initial response to imatinib (Cools et al. 2003a). A mutation of the threonine at position 674, (analogous to the T315I mutation of BCR/ABL), located in the ATP-binding region of the PDGFRα protein, confers FIP1L1/PDGFRα resistance to imatinib in *in vitro* and *in vivo* models of myelo- and lympho-proliferation (Cools et al. 2003a; Stover et al. 2005). Intracellular signaling of FIP1L1/PDGFRα has been investigated in transfected Ba/F3 cells, an IL-3-dependent hematopoietic cell line. After transfection with FIP1L1/PDGFRα, these cells demonstrate IL-3 independent growth and intense activation of the transcriptional factor STAT5 (Cools et al. 2003a; Stover et al. 2006). A similar process occurring in EoL-1 cells (Cools et al. 2003a; Verstovsek et al. 2006) which endogenously express the *FIP1L1/PDGFRA* fusion gene (Cools et al. 2004; Griffin et al. 2003). STAT5-phosphorylation is inhibited by imatinib in a dose-dependent manner, whereas it was resistant to imatinib in FIP1L1/PDGFRα (T674I)-expressing Ba/F3 cells (Cools et al. 2003a). STAT3 and STAT5 transcriptional factors would appear to be activated either directly by FIP1L1/PDGFRα or through interaction with Janus activated kinase (JAK) (Li et al. 2005; Zhang et al. 2004). Notably, it has been recently shown that JAK2 and STAT3/STAT5 are upregulated in FIP1L1/PDGFRα-expressing primary granulocytes and rapidly down-regulated by imatinib treatment (Li et al. 2005) (Fig. 2). In contrast to the STAT5 pathway, the involvement of MAPK pathway in FIP1L1/PDGFRα fusion protein signaling is still controversial. Early studies on the downstream signaling of FIP1L1/PDGFRα showed that, unlike other activated tyrosine kinases, FIP1L1/PDGFRα does not appear to induce ERK1/2 activation (Cools et al. 2003a). However, a more recent study showed that ERK1/2 is indeed a downstream target of FIP1L1/PDGFRα in both FIP1L1/PDGFRα expressing Ba/F3 and EoL-1 cell lines (Lierman et al. 2006).

RTKs such as PDGFR and epidermal cell growth factor receptor (EGFR) can be transactivated through some G-protein-coupled receptors, without ligand-RTK interaction (Daub et al. 1996; Herrlich et al. 1998). In the same way, the transactivation of EGFR through the chemokine receptor 3 (CCR3), a receptor for eosinophil selective chemokines (eotaxins) has been recently reported in epithelial cells (Adachi et al. 2004). An intriguing possibility is that the CCR3-MAP kinase activation pathway in eosinophils may be modulated through RTK activation, specifically PDGFR. It has been shown that ERK phosphorylation and eotaxin-induced chemotaxis of primary eosinophils are specifically inhibited by the PDGFR-inhibitor AG1295 (Adachi et al. 2006). These authors also demonstrated that, although at low levels, both PDGFRα and PDGFRβ are endogenously expressed in eosinophils (Adachi et al. 2006). Taken together,

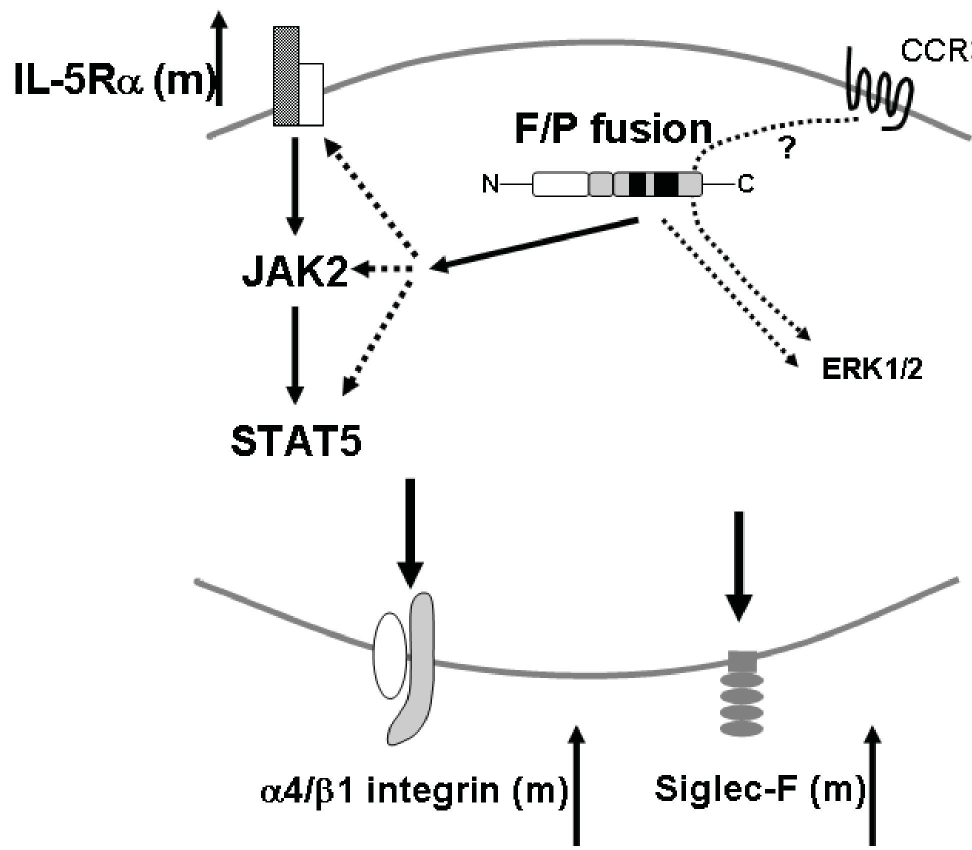

Figure 2. FIP1L1/PDGFRα^+ eosinophil signaling.
FIP1L1/PDGFRα^+ primary eosinophils express upregulated IL-5 receptor (IL-5Rα) and JAK2/STAT5 pathway may be involved in FIP1L1/PDGFRα induced disease development(Li et al. 2005; Yamada et al. 2006; Zhang et al. 2004). FIP1L1/PDGFRα may phosphorylate ERK1/2 though its direct signal or indirect signal such as transactivation(Adachi et al. 2006; Lierman et al. 2006). There is a possibility that CCR3/ERK1/2 pathway is amplified by FIP1L1/PDGFRα^+(Adachi et al. 2006). It is speculated that the integrated signaling may upregulate the expressions α4 integrin and Siglec-F (Yamada et al. 2006).

these findings imply that FIP1L1/PDGFRα fusion may also modify the CCR3-MAP kinase pathway and the eosinophil functions dependent on that pathway (Fig. 2).

FIP1L1/PDGFRα-induced myeloproliferative disease models

Cools and colleagues reported a murine model of disease induced after BM transplantation of *FIP1L1/PDGFRA*-transduced HSC/P (Cools et al. 2003b). The introduction of the *FIP1L1/PDGFRA* fusion gene by itself into BM HSC/P induced a myeloproliferative disorder. The disease was characterized by severe leukocytosis with mild eosinophilia (5–20%) in the peripheral blood and myeloid tissue infiltration, mostly neutrophil infiltration in multiple organs similar to that found in p210-BCR/ABL induced CML-like disease. The disease development was completely inhibited by imatinib. Several groups have also determined the response of FIP1L1/PDGFRα-induced myeloproliferative disease to the tyrosine kinase inhibitors, PKC412 (Cools et al. 2003b) and nilotinib (Stover et al. 2005; von Bubnoff et al. 2006) in this murine model, demonstrating that both inhibitors were efficient in preventing disease development. This was clinically interesting, as the possibility of imatinib-resistant mutations has been reported (Cools et al. 2003a; Ohnishi et al. 2006). FIP1L1/PDGFRα expression alone induced a moderate eosinophilia, which did not fully resemble human HES/CEL. Indeed, we have also found that FIP1L1/PDGFRα expression preferentially induces eosinophilia when compared to proliferation of other myeloid lineages, but is not sufficient to develop result in severe blood and tissue eosinophilia resembling human HES/CEL in the mouse (Yamada et al. 2006). This might be due to specific differences between human and murine hematopoiesis, or to

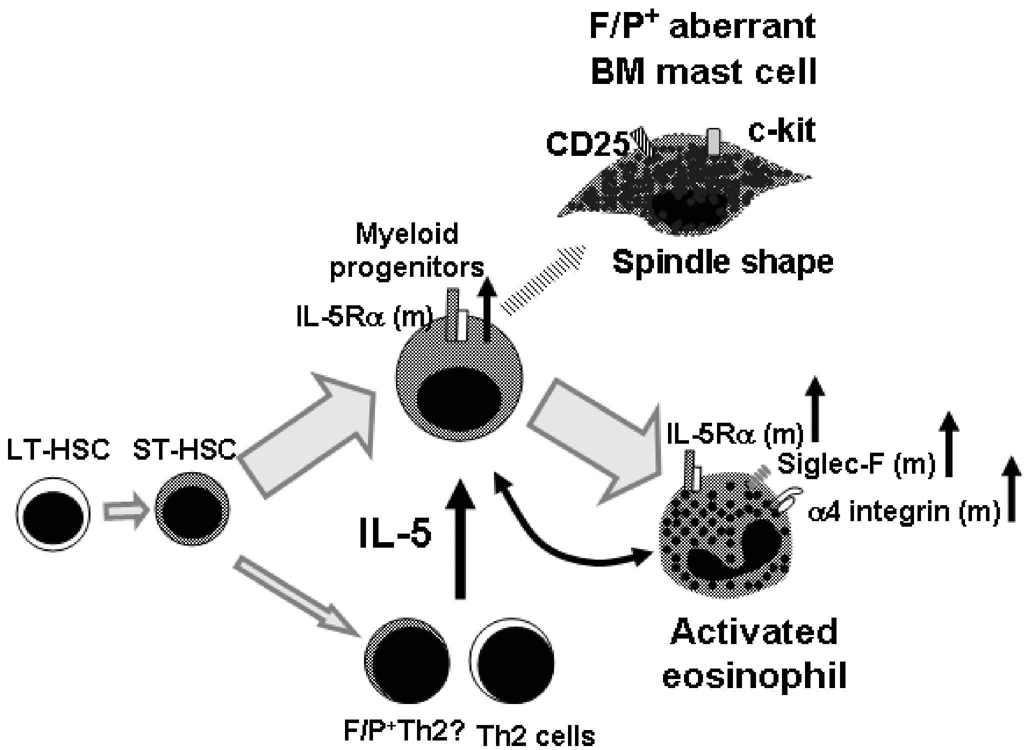

Figure 3. FIP1L1/PDGFRα fusion promotes aberrant eosinophil and mast cell development synergized with IL-5 signaling. FIP1L1/PDGFRα fusion would occur in short term repopulating stem cells or early progenitors and then differentiated into not only FIP1L1/PDGFRα^+ myeloid cells but also FIP1L1/PDGFRα^+ lymphoid cells (Robyn et al. 2006; Tefferi et al. 2004; Yamada et al. 2006). The characterization of FIP1L1/PDGFRα^+ lymphocytes has not been performed but there are possibilities that FIP1L1/PDGFRα preferentially differentiated lymphoid cells into IL-5- producing Th2 cells, or other highly IL-5 producing Th2 cells may be involved. IL-5Rα expression and frequency of IL5Ra-expressing cells are specifically increased in FIP1L1/PDGFRα^+ cells (Yamada et al. 2006), suggesting that IL-5 response in FIP1L1/PDGFRα^+ cells may be upregulated. Mature FIP1L1/PDGFRα^+ eosinophils demonstrate upregulation of α4 integrin and Siglec-F indicating the eosinophilis would be activated (Yamada et al. 2006). In addition to eosinophils, FIP1L1/PDGFRα^+ aberrant mast cells, which have spindle shape and express CD25/ckit, have been observed in bone marrow of FIP1L1/PDGFRα^+ patients (Klion et al. 2003).

the presence in HES/CEL of secondary events that are needed to facilitate the development of hypereosinophilia (Figure 3).

A subgroup of patients with HES displays aberrant, sometimes clonal, $CD3^-/CD4^+$ Th2 lymphocytes which secrete large amounts of IL-5 (Bank et al. 2001) where anti-IL-5 treatment has been shown to be effective (Garrett et al. 2004; Plotz et al. 2003). In some patients, elevated IL-5 levels are not detected in circulation, even in patients with HES that respond to anti-IL-5 treatment (Garrett et al. 2004; Klion et al. 2004a; Owen et al. 1989), indicating that paracrine (Simon et al. 1999) and autocrine (Lamkhioued et al. 1996) effects of IL-5 produced by local T-cells and/or eosinophils may have critical roles in HES. Serum IL-5 levels have also been reported to be elevated in imatinib-responder HES patients including FIP1L1/PDGFRα^+ HES/CEL patients (Pardanani and Tefferi, 2004). In addition to IL-5 levels, there is a possibility that FIP1L1/PDGFRα^+ lymphocytes may affect the disease development in humans since it has been demonstrated that a subpopulation of $CD3^+$ cells in patients with FIP1L1/PDGFRα^+-expressing HES/CEL also express FIP1L1/PDGFRα (Robyn et al. 2006; Tefferi et al. 2004). Interestingly, a T-cell-dependent murine model of IL-5 overexpression (induced by the CD2-IL-5 transgenic) is associated with blood eosinophilia but not tissue eosinophilia (Dent et al. 1990). Since neither IL-5 nor FIP1L1/PDGFRα overexpression alone induce substantial tissue eosinophilia, is possible that the combination of two or more events may be needed to induce the development of HES/CEL-like disease (Fig. 3). Clinical studies that combine imatinib and anti-IL-5 therapies have not been reported, but may represent an attractive approach to the therapy of FIP1L1/PDGFRα-positive CEL.

These collective findings motivated us to develop a FIP1L1/PDGFRα-induced disease model in the presence of T-cell-dependent IL-5 overexpression. In order to determine if the expression of the *FIP1L1/PDGFRA* fusion gene in the presence of T-cell IL-5 overexpression induces HES-like disease in mice, lethally irradiated wild-type mice were transplanted with FIP1L1/PDGFRα-transduced HSC/P derived from CD2-IL-5 Tg mouse BM (Yamada et al. 2006). These mice developed a rapidly progressive phenotype featuring intense leukocytosis, hepatosplenomegaly, strikingly high eosinophilia and eosinophilic infiltration of non-hematopoietic as well as hematopoietic tissues, resembling human HES (CEL-like mice). The eosinophils in FIP1L1/PDGFRα induced CEL-like mice expressed increased levels of surface alpha 4-integrin and Siglec-F, two molecules that are involved in eosinophil activation (Fig. 2). In addition, the level of expression and frequency of IL-5Rα^+ cells was increased in FIP1L1/PDGFRα^+ splenocytes. IL-5Rα expression was not upregulated in p210-BCR/ABL fusion positive cells, demonstrating FIP1L1/PDGFRα specificity for the IL-5 pathway (Yamada et al. 2006). Since both IL-5R and FIP1L1/PDGFRα fusion protein can activate the JAK2/STAT5 pathway (Buitenhuis et al. 2003; Cools et al. 2003a; de Groot et al. 1998), this may be the mechanism by which the combination of FIP1L1/PDGFRα expression and the overexpression of IL-5 converge at the JAK/STAT signaling pathway and triggering a CEL-like disease. This effect would be noteworthy since FIP1L1/PDGFRα may amplify the IL-5 signaling through upregulation of receptor expression (Fig. 2).

In order to determine if the murine CEL-like disease model represents a stem cell or early progenitor-derived proliferative disease, secondary transplantation from diseased mice was performed (Yamada et al. 2006). When a high number of splenocytes from primary CEL-like mice was transplanted into lethally-irradiated secondary recipients, mice developed a similar CEL-like disease, and at the same time, demonstrated FIP1L1/PDGFRα expression in peripheral blood B- and T-cells for up to 7 weeks after secondary transplantation. These data strongly suggest the involvement of a short-term repopulating stem cell or an early myeloid progenitor (Fig. 3).

A major question is whether the combination of IL-5 overexpression together with other fusion genes may induce the same type of hypereosinophilia. This is underscored by the fact that human *p210-BCR/ABL*-induced CML is sometimes associated with hypereosinophilia in the context of a general chronic myeloproliferative disorder (eosinophilic variant of CML) (Bennett et al. 1994; Gotlib et al. 2000). Therefore, we introduced the exogenous expression of the *p210-BCR/ABL* fusion gene in HSC/P and tested it against the *in vivo* effect of FIP1L1/PDGFRα fusion protein in the presence of IL-5 overexpression. Notably, *p210-BCR/ABL*, in the presence of IL-5 overexpression induced eosinophilia at significantly lower levels than that induced by FIP1L1/PDGFRα. Moreover, unlike FIP1L1/PDGFRα-expressing splenocytes (Yamada et al. 2006); p210-BCR/ABL$^+$ cells had no upregulation of IL-5Rα expression (Yamada et al. 2006).

Summary

The FIP1L1/PDGFRα fusion protein has provoked great attention since it was described as a cause of HES/CEL. The role of FIP1L1/PDGFRα in leukemogenesis, through its constitutively active tyrosine kinase activity, has been demonstrated in *in vitro* and *in vivo* models. However, in terms of the role of specific hypereosinophilia, it has only recently been shown that FIP1L1/PDGFRα is specifically associated with the development of hypereosinophilia *in vivo* when combined with IL-5 overexpression (Figures 2 and 3). Although, the mechanism behind the aberrant eosinophilopoiesis induced by FIP1L1/PDGFRα in patients remains elusive, this finding suggests that the blocking of IL-5 pathway such as anti-IL-5 treatment would be effective for intrinsic HES including FIP1L1/PDGFRα^+ CEL as well as IL-5-producing T-lymphocyte-associated HES/CEL. Further elucidation of the specific mechanisms by which FIP1L1/PDGFRα induces CEL will shed light on the development of new molecular therapies for CEL/HES and probably, other eosinophilic disorders.

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