TABLE 1
Peripheral blood examinations 16 weeks after transplantation

Mouse	White blood cells × 10^9/liter	Eosinophil
		%
Mock-1	88.3	1.2
Mock-2	96.2	2.4
Mock-3	83.5	2.6
Mock-4	102.2	0.8
Mock-5	88.2	2
FIP1L1-PDGFRα-1	563.2	3.6
FIP1L1-PDGFRα-2	121.1	1.2
FIP1L1-PDGFRα-3	492.3	4.8
FIP1L1-PDGFRα-4	140.1	3.6
FIP1L1-PDGFRα-5	662.3[a]	0.2

[a] CD3(+)CD8(+) cells: 96%.

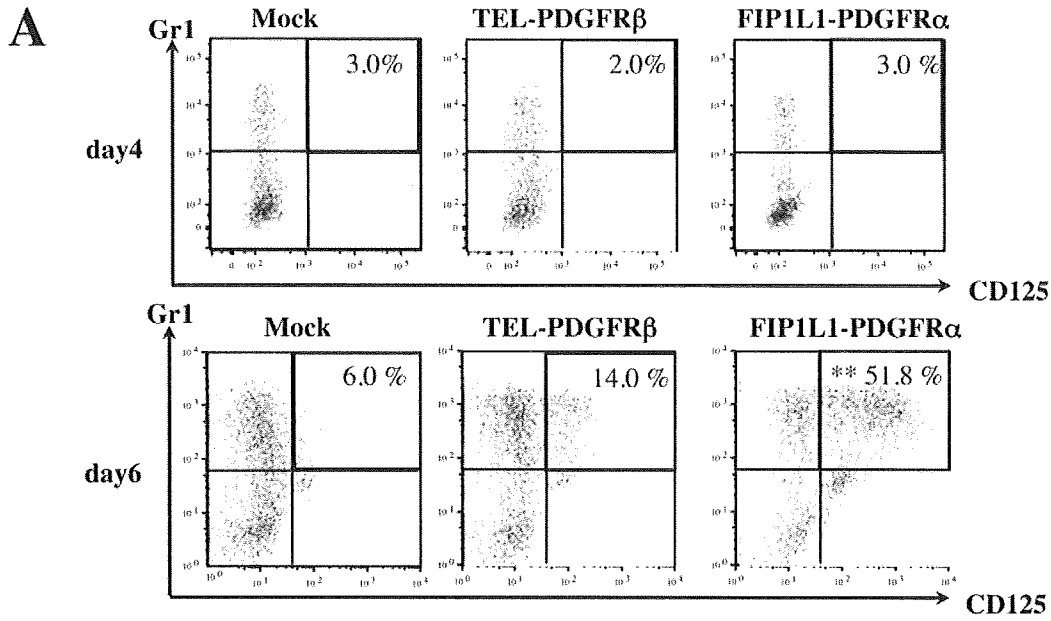
still kept colony-forming activities even after the third and fourth plating (FIP1L1-PDGFRα *versus* mock at the third plating, $p < 0.01$; at the fourth plating, $p < 0.01$), although these activities were rather reduced (Fig. 1*C*, *left*). On the other hand, even if FIP1L1-PDGFRα or TEL-PDGFRβ was introduced, CMPs could not form any colony at the third plating, as was the case with mock-infected CMPs (Fig. 1*C*, *right*). To evaluate leukemogenic potential of FIP1L1-PDGFRα-transduced KSLs *in vivo*, we transplanted these cells into lethally irradiated mice in combination with freshly prepared competitor KSLs. As a result, although none of the mice transplanted with mock-transduced KSLs developed leukemia or MPD, FIP1L1-PDGFRα-transduced KSLs developed MPD in three mice and acute leukemia in one mouse of five recipient mice within 15 weeks after transplantation (Table 1). However, in agreement with the previous report (16), none of the five recipient mice developed eosinophilic disorders. In addition, none of the 10 mice transplanted with FIP1L1-PDGFRα-transduced CMPs developed MPD or leukemia (data not shown). Together, these results indicate that FIP1L1-PDGFRα can confer the ability of cytokine-independent growth/survival on KSLs and enhance their self-renewal, whereas it cannot immortalize CMPs *in vitro* or *in vivo*.

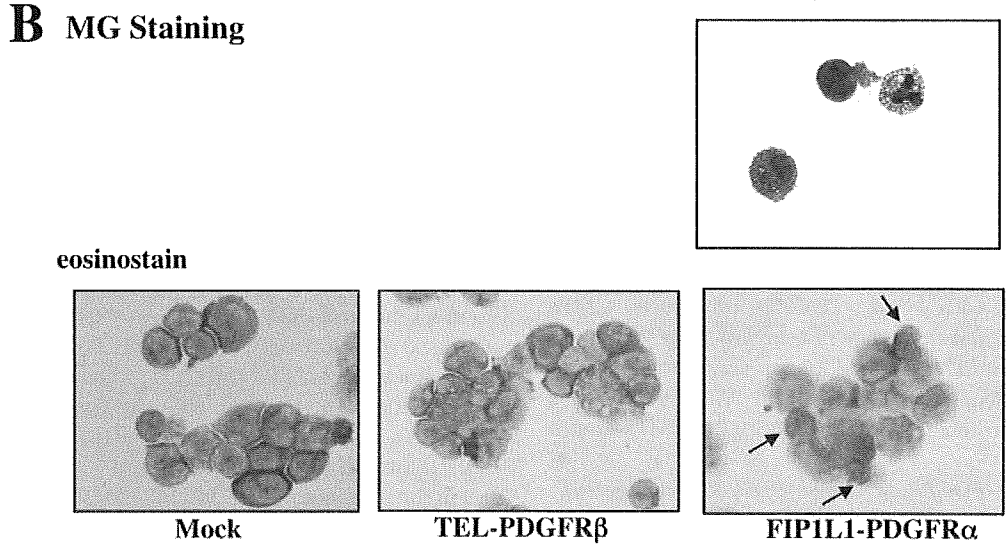
Effects of FIP1L1-PDGFRα and TEL-PDGFRβ on Differentiation from KSLs—We next investigated whether FIP1L1-PDGFRα or TEL-PDGFRβ influences the lineage commitment and subsequent differentiation of KSLs. For this purpose, we infected retrovirus harboring FIP1L1-PDGFRα or TEL-PDGFRβ into KSLs; cultured them with SCF, TPO, FLT3L, and IL-6; and examined the expression of a granulocyte marker (Gr-1) and an eosinophil marker (IL-5 receptor α, CD125) in GFP-positive cells by flow cytometry. After 4-day cultures, there was not an apparent difference in the expression pattern of these markers among FIP1L1-PDGFRα-, TEL-PDGFRβ-, and mock-transduced KSLs (Fig. 2*A*, *top*). However, after 6-day cultures, TEL-PDGFRβ- or FIP1L1-PDGFRα-transduced KSLs yielded significantly increased Gr-1^+ fraction (66.8 and 77.5%, respectively) compared with mock-transduced KSLs (49.6%). In addition, it was of particular interest that 51.8% of FIP1L1-PDGFRα-transduced KSLs grew to express CD125 and Gr-1 simultaneously, whereas only 6.0% of mock-transduced and 14.0% of TEL-PDGFRβ-transduced KSLs revealed this phenotype (FIP1L1-PDGFRα *versus* mock, $p < 0.01$; Fig. 2*A*, *bottom*). These results imply that FIP1L1-PDGFRα but not TEL-PDGFRβ preferentially imposes the commitment and differentiation to the eosinophilic lineage.

To examine whether Gr-1^+CD125^+ cells that developed from FIP1L1-PDGFRα-transduced KSLs are actually eosinophil precursors, we further cultured these KSLs with a cytokine mixture containing IL-5 for an additional 5 days. As a result, most of FIP1L1-PDGFRα-transduced but not mock- or TEL-PDGFRβ-transduced KSLs came to possess large granule characteristics of mature eosinophil in the MG staining, which were positive for the eosinostain (Fig. 2*B*). Furthermore, after 10-day cultures, we examined the mRNA expression of eosinophil-related genes, *GATA-1*, *IL-5Rα*, and *C/EBPε*, by RT-PCR analysis using sorted GFP-positive cells. As shown in Fig. 2*C*, *IL-5Rα* and *C/EBPε* mRNAs were detected only in FIP1L1-PDGFRα-transduced KSLs. Also, *GATA-1* mRNA was more intensively expressed in FIP1L1-PDGFRα-transduced KSLs than in mock- or TEL-PDGFRβ-transduced KSLs. These data indicate that Gr-1^+CD125^+ cells that developed from FIP1L1-PDGFRα-transduced KSLs can indeed differentiate into mature eosinophils.

Effects of FIP1L1-PDGFRα on Differentiation of CMPs, MEPs, and CLPs—It was previously shown that eosinophil precursors stochastically develop from HSCs through MMP, CMP, and GMP (40, 41). Therefore, at first, we examined whether FIP1L1-PDGFRα can enhance the development of eosinophils from CMPs. For this purpose, we isolated CMPs from murine BM mononuclear cells by FACS using several markers (Fig. 3*A*). Then we introduced FIP1L1-PDGFRα into these cells and cultured them with SCF, IL-6, FLT3L, and TPO for 6 days. As was the case with KSLs, FIP1L1-PDGFRα remarkably enhanced the development of Gr-1^+CD125^+ cells from CMPs compared with mock cultures (57% *versus* 6%, $p < 0.01$; Fig. 3*B*).

Our next question was whether FIP1L1-PDGFRα could convert the lineages of MEPs and CLPs, which were already committed to the other lineages, into the eosinophil lineage. To address this issue, we introduced FIP1L1-PDGFRα or TEL-PDGFRβ into MEPs. When cocultured with a stroma cell line OP-9 in the presence of SCF and EPO for 9 days, 58% of mock-infected and 41% of TEL-PDGFRβ-infected MEPs came to reveal the Ter119^+CD125^- erythroid phenotype. In contrast, only 26% of FIP1L1-PDGFRα-infected MEPs revealed this phenotype (FIP1L1-PDGFRα *versus* mock, $p < 0.05$; Fig. 3*C*, *top*). Moreover, 50% of FIP1L1-PDGFRα-transduced MEPs differentiated into CD125^+Gr-1^+ cells, whereas only 16% of mock-infected and 14% of TEL-PDGFRβ-infected MEPs revealed this phenotype (FIP1L1-PDGFRα *versus* mock, $p < 0.01$; Fig. 3*C*, *bottom*). Similarly, after 9-day cultures in serum-free medium supplemented with TPO and IL-11, although mock-transduced MEPs effectively gave rise to CD41^+Gr-1^- cells (17%), only 2% of FIP1L1-PDGFRα-infected MEPs revealed this phenotype (FIP1L1-PDGFRα *versus* mock, $p < 0.01$; Fig. 3*D*). Also, mock-transduced MEPs were found to become large polyploid megakaryocytes in morphological analysis, whereas most of the FIP1L1-PDGFRα-transduced MEPs remained small and mononuclear (Fig. 3*E*). Together, these results indicate that FIPIL1-PDGFRα inhibits erythroid and megakaryocytic differentiation from MEPs and imposes lineage conversion to the eosinophil lineage.





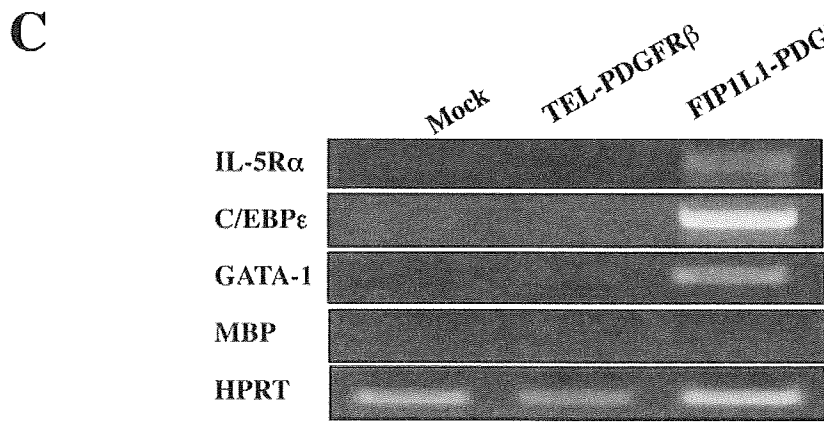


FIGURE 2. **Eosinophil development from KSLs.** *A*, after retrovirus transduction, KSLs were cultured with SCF, TPO, IL-6, and FLT3L, and FACS analysis was performed after 4 days (*top*) and 6 days (*bottom*). GFP^+ cells were gated, and the expression of Gr-1 and CD125 was analyzed. **, $p < 0.01$ compared with the value of mock-transduced cells ($n = 3$). *B*, after 6-day cultures with SCF, TPO, IL-6, and FLT3L, retrovirus-infected KSLs were further cultured with a cytokine mixture containing IL-5 for 5 days. Transduced cells were subjected to May-Giemsa staining (*top*) and eosinostain (*bottom*). *C*, after 10-day cultures with TPO, IL-6, FLT3L, and SCF, GFP-positive cells were sorted, and the expression of eosinophil-related genes was analyzed by RT-PCR analysis.

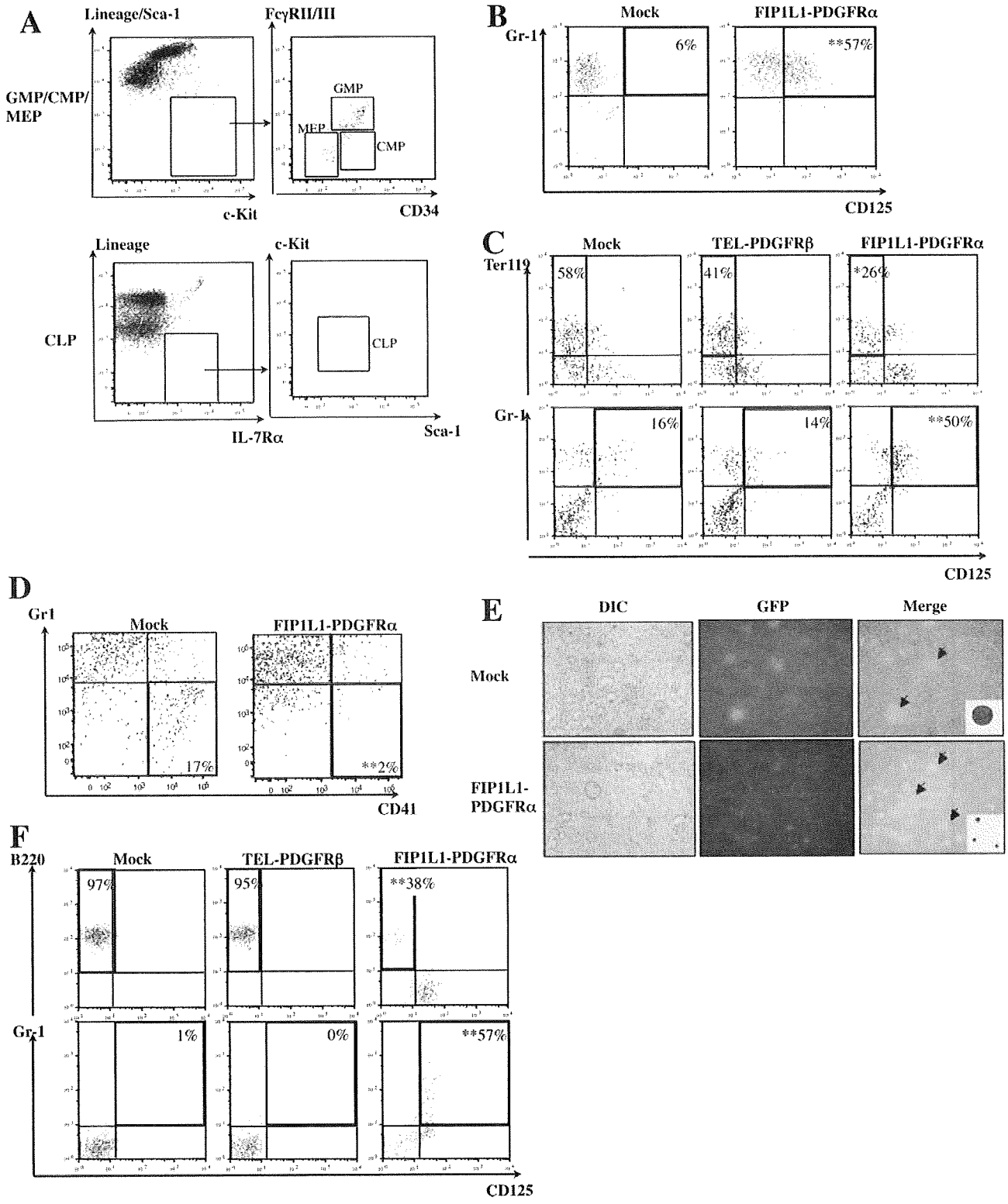


FIGURE 3. **FIP1L1-PDGFRα-induced eosinophil development from CMPs, MEPs, and CLPs.** *A*, isolation of GMPs/CMPs/MEPs and CLPs from murine mononuclear cells by a FACS using several markers. *B*, mock-, FIP1L1-PDGFRα-, or TEL-PDGFRβ-transduced CMPs were cultured with SCF, IL-6, FLT3L, and TPO for 6 days. Then the expression of CD125 and Gr-1 was analyzed by flow cytometry. *C*, mock-, FIP1L1-PDGFRα-, or TEL-PDGFRβ-transduced MEPs were cocultured with OP-9 cells in the presence of EPO and SCF for 8 days and then subjected to FACS analysis. *D* and *E*, mock- or FIP1L1-PDGFRα-transduced MEPs were cultured in serum-free medium supplemented with TPO and IL-11 for 9 days and subjected to FACS analysis. Transduced cells were observed with differential interference contrast (*DIC*) and fluorescence microscopy. Mock- and FIP1L1-PDGFRα-transduced GFP-positive cells (*arrows*) were sorted and subjected to May-Giemsa staining. *F*, retrovirus-transduced CLPs were cocultured with OP-9 cells in the presence of SCF, IL-7, and FLT3L for 2 days. Then granulocyte-macrophage colony-stimulating factor was added into the medium, and cells were cultured for an additional 8 days. **, $p < 0.01$ compared with the value of mock-transduced cells ($n = 3$).

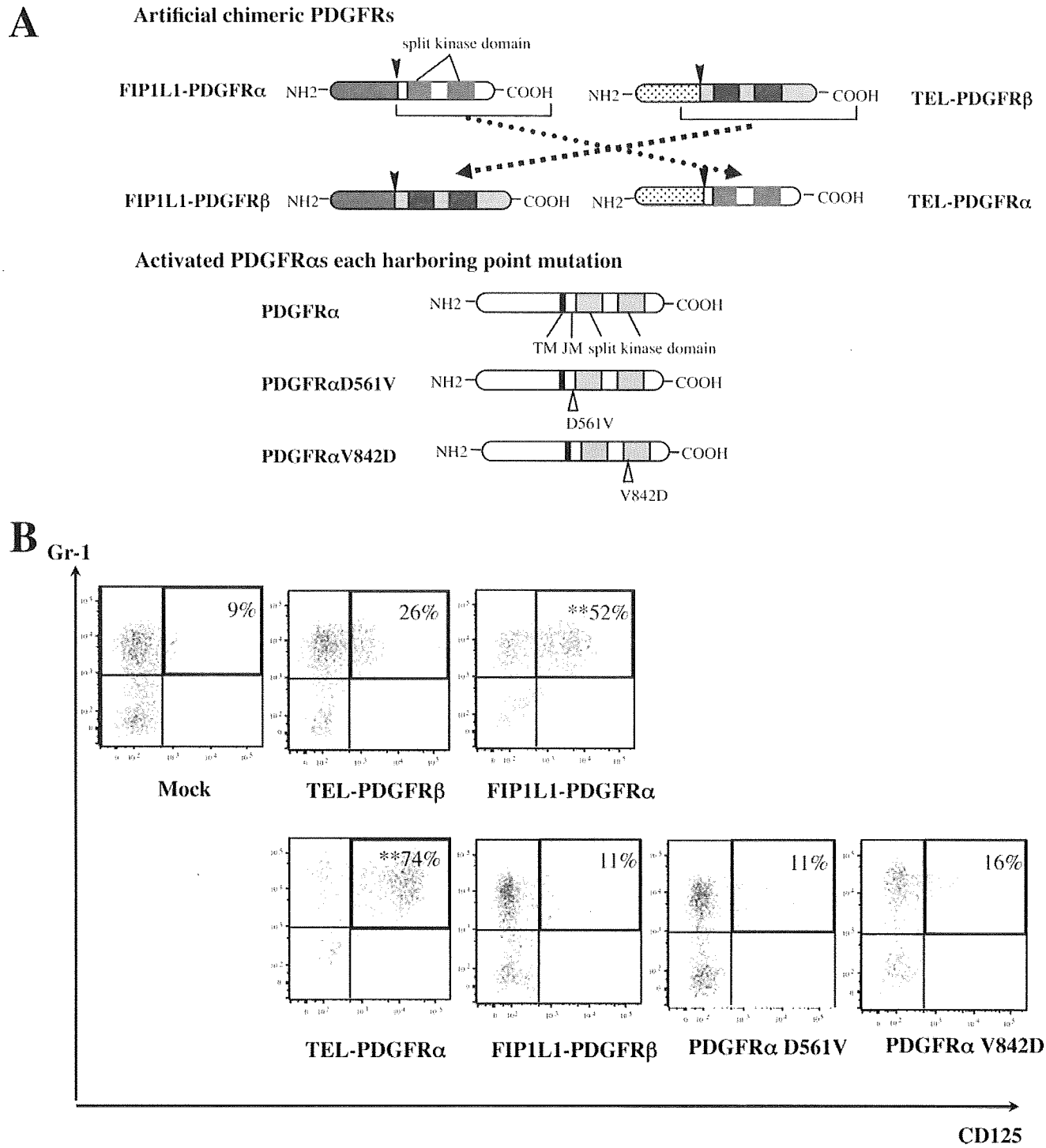


FIGURE 4. **Function of FIP1L1 and PDGFRα in FIP1L1-PDGFRα-induced eosinophil development.** *A*, schematic representation of FIP1L1-PDGFRβ and TEL-PDGFRα, PDGFRαD561V, and PDGFRαD842V. In FIP1L1-PDGFRβ and TEL-PDGFRα, FIP1L1 in FIP1L1-PDGFRα and TEL in TEL-PDGFRβ were completely exchanged one another. Splicing sites are indicated with *black arrows*, and point mutation sites are indicated with *vacant arrows*. *TM*, transmembrane domain; *JM*, juxtamembrane domain. *B*, murine KSLs were infected with the retrovirus, as indicated, and cultured with SCF, TPO, IL-6, and FLT3L for 6 days. Then expression of CD125 and Gr-1 was analyzed by flow cytometry. **, $p < 0.01$ compared with the value of mock-transduced cells ($n = 3$).

Next, we introduced FIP1L1-PDGFRα into CLPs and cocultured them with OP-9 cells in the presence of SCF, IL-7, and FLT3L. After 10-day cultures, 97% of mock- and 95% of TEL-PDGFRβ-transduced CLPs came to have the $B220^+CD125^-$ B-lymphoid phenotype, whereas only 38% of FIP1L1-PDGFRα-transduced CLPs had this phenotype (FIP1L1-PDGFRα *versus* mock, $p < 0.01$). Furthermore, a considerable proportion of FIP1L1-PDGFRα-transduced CLPs but

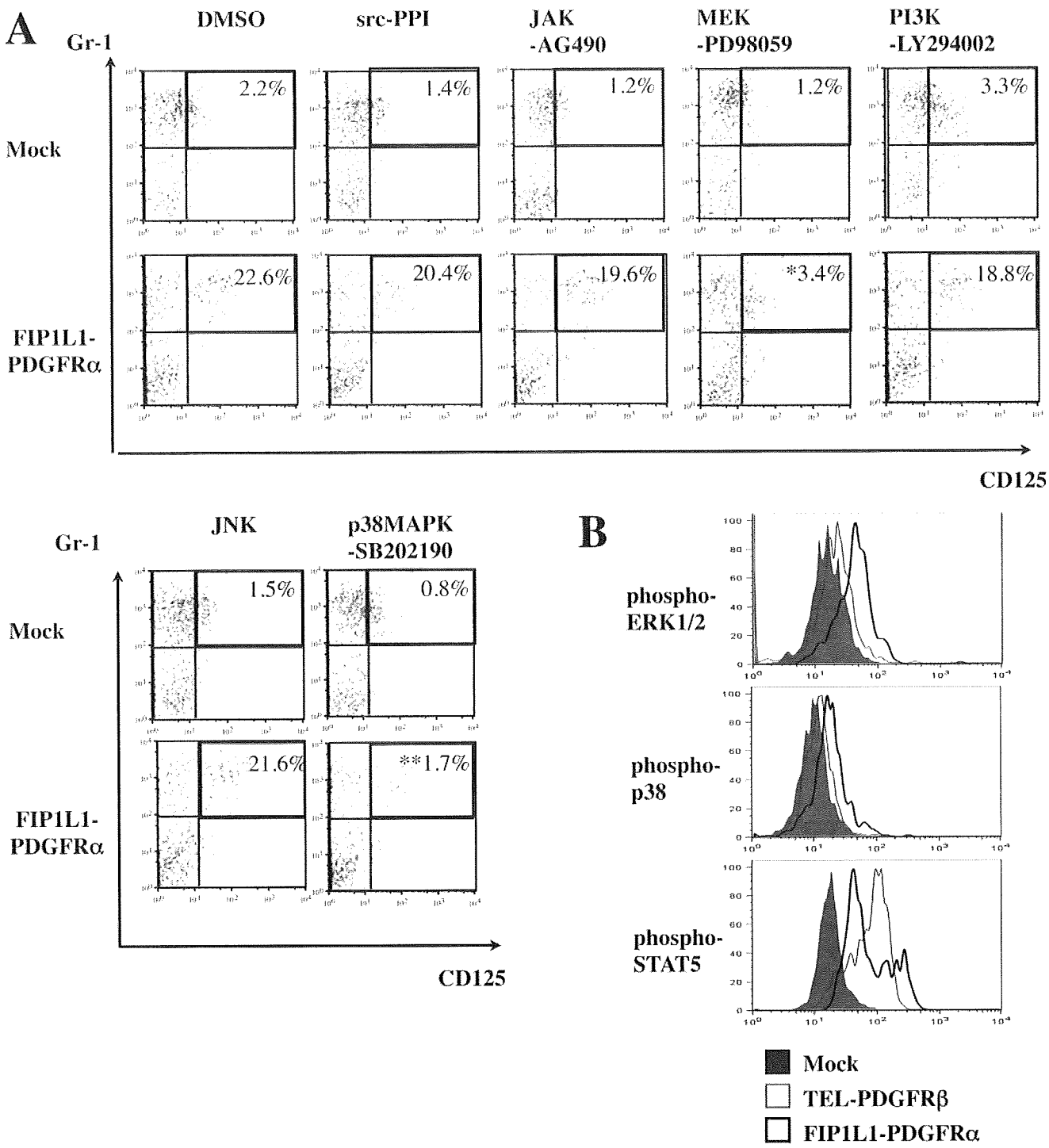
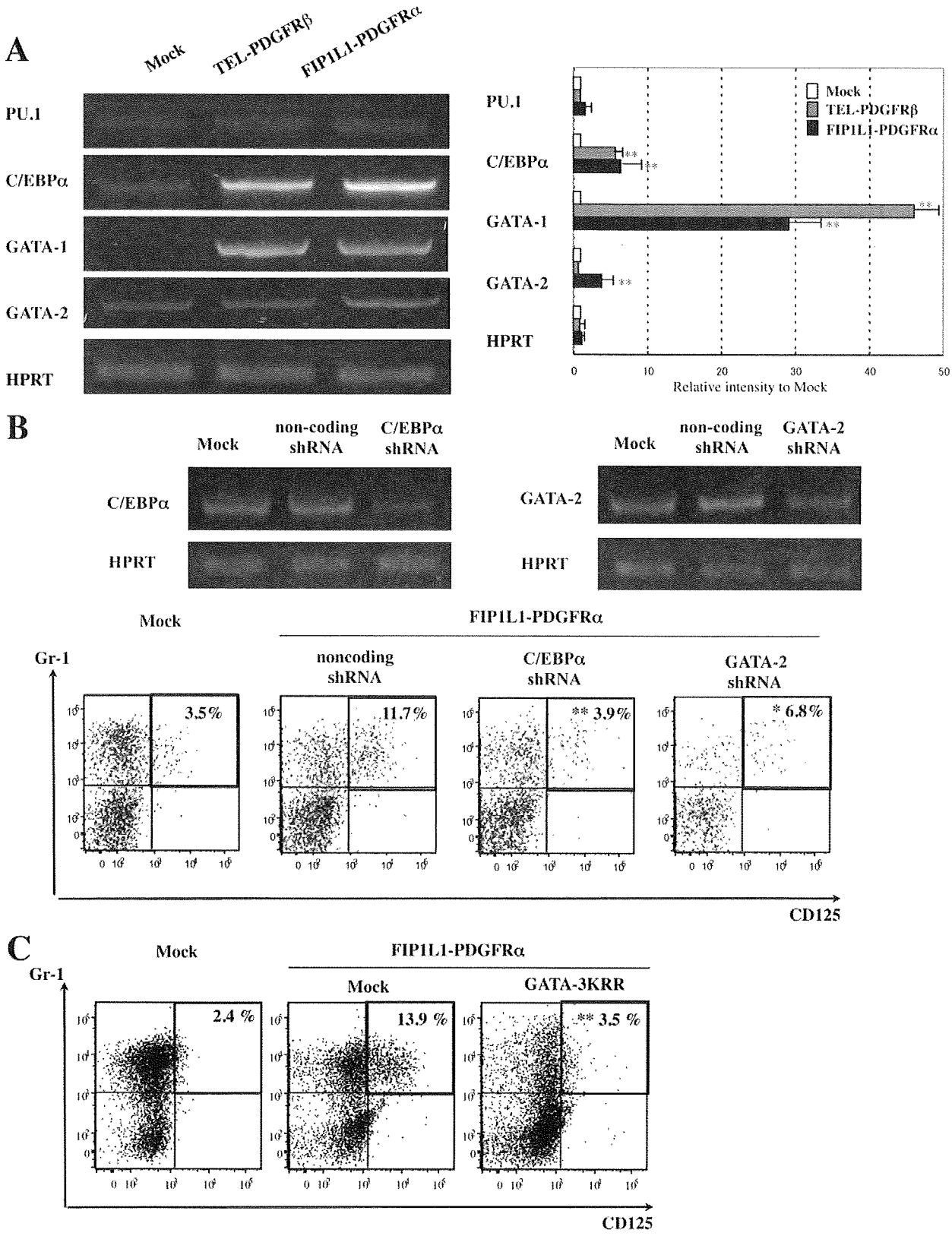


FIGURE 5. **Roles of signal cascades in FIP1L1-PDGFRα-induced eosinophil development.** *A*, murine KSLs were infected with the retrovirus indicated and cultured with SCF, TPO, IL-6, and FLT3L with or without kinase inhibitors as indicated and then subjected to FACS analysis. *, $p < 0.05$; **, $p < 0.01$ compared with the value of DMSO-treated cells ($n = 4$). *B*, FIP1L1-PDGFRα-, TEL-PDGFRβ-, or mock- transduced KSLs were cultured for 2 days, and the phosphorylation status of ERK1/2, p38MAPK, and STAT5 was analyzed using Phosflow technology.

not mock- or TEL-PDGFRβ-transduced CLPs aberrantly differentiate into Gr-1$^+$CD125$^+$ cells (percentage of Gr-1$^+$CD125$^+$ cells as follows: FIP1L1-PDGFRα, 57% *versus* mock (1%) ($p < 0.01$); TEL-PDGFRβ, 0% (Fig. 3*F*). We further cultured Gr-1$^+$CD125$^+$ cells that developed from FIP1L1-PDGFRα-transduced CLPs with a cytokine mixture containing IL-5 and confirmed that these cells became positive for eosinostain (data not shown). These results indicate that FIP1L1-PDGFRα inhibits B-lymphoid differentiation from CLPs and instructs them to differentiate into the eosinophil lineage.

Function of FIP1L1 and PDGFRα in the Fusion Protein—It was previously shown that the FIP1L1 moiety is dispensable

for kinase activation and for transforming properties of FIP1L1-PDGFRα (42). To determine the role of FIP1L1 in FIP1L1-PDGFRα-enhanced eosinophil development, we generated two artificial chimeric constructs, *FIP1L1-PDGFRβ* and *TEL-PDGFRα*, in which *FIP1L1* in *FIP1L1-PDGFRα* and *TEL* in *TEL-PDGFRβ* were completely replaced (Fig. 4*A*). In addition,



we generated retrovirus vectors for constitutively active PDGFRα (PDGFRαV561D and PDGFRαD842V), which are considered to be causative mutations of gastrointestinal stromal tumors (43) (Fig. 4*A*). When expressed in a murine IL-3-dependent cell line, Ba/F3, all of the four PDGFR mutants conferred IL-3-independent growth on these cells (data not shown). Also, Western blot analysis demonstrated that these PDGFR mutants phosphorylated various cellular proteins, including themselves (data not shown), indicating that these proteins act as constitutively active tyrosine kinases.

We transduced these retrovirus expression vectors into KSLs and cultured them with SCF, TPO, FLT3L, and IL-6 for 6 days. As shown in Fig. 4*B*, only TEL-PDGFRα and not FIP1L1-PDGFRβ, PDGFRαV561D, or PDGFRαD842V promoted eosinophil development from KSLs (percentage of Gr-1$^+$CD125$^+$ fraction as follows: TEL-PDGFRα, 74%; FIP1L1-PDGFRβ, 11%; PDGFRαV561D, 11%; PDGFRαD842V, 16%) (TEL-PDGFRα *versus* mock, $p < 0.01$) (Fig. 4*B*), indicating that FIP1L1 is dispensable for FIP1L1-PDGFRα-mediated eosinophil development and that PDGFRα-mediated signaling but not PDGFRβ-mediated signaling is required for inducing eosinophil development. However, because neither PDGFRαV561D nor PDGFRαD842V promoted eosinophil development, specific kinase activity transmitted from chimeric PDGFRα was supposed to be necessary to enhance eosinophil development.

Both a MEK1/2 Inhibitor and a p38^{MAPK} Inhibitor Blocked FIP1L1/PDGFRα-induced Eosinophil Development from KSLs—PDGFRα activates various downstream cascades, thereby exerting its biologic activity (44). To seek out the mechanism underlying instructive eosinophil differentiation induced by FIP1L1-PDGFRα, FIP1L1-PDGFRα- or mock-transduced KSLs were cultured with or without several kinase inhibitors as indicated (Fig. 5*A*).

As shown in Fig. 5*A* (*top*), neither a c-Jun N-terminal kinase inhibitor, a phosphatidylinositol 3-kinase inhibitor (LY294002), an Src inhibitor (PPI), nor a JAK2/STAT inhibitor (AG490) influenced FIP1L1-PDGFRα-enhanced eosinophil development, since about 20% of cells came to be CD125$^+$Gr1$^+$ after 5-day cultures as was seen after the culture without an inhibitor (Fig. 2*A*). In contrast, a MEK inhibitor (PD98059) and a p38^{MAPK} inhibitor (SB202190) reduced the CD125$^+$Gr1$^+$ fraction to 3.4% ($p < 0.05$) and 1.7% ($p < 0.01$), respectively (Fig. 5*A*, *bottom*). We also analyzed the phosphorylation states of ERK, STAT5, and p38^{MAPK} in FIP1L1-PDGFRα- or TEL-PDGFRβ-transduced KSLs by flow cytometry. As shown in Fig. 5*B*, ERK1/2 and p38MAPK but not STAT5 were phosphorylated more intensely in FIP1L1-PDGFRα-transduced KSLs than in mock- or TEL-PDGFRβ-transduced KSLs. These data suggest that FIP1L1-PDGFRα instructs HSCs/HPCs to differentiate into eosinophil progenitors through the activation of MEK1/2-ERK1/2 and p38 pathways.

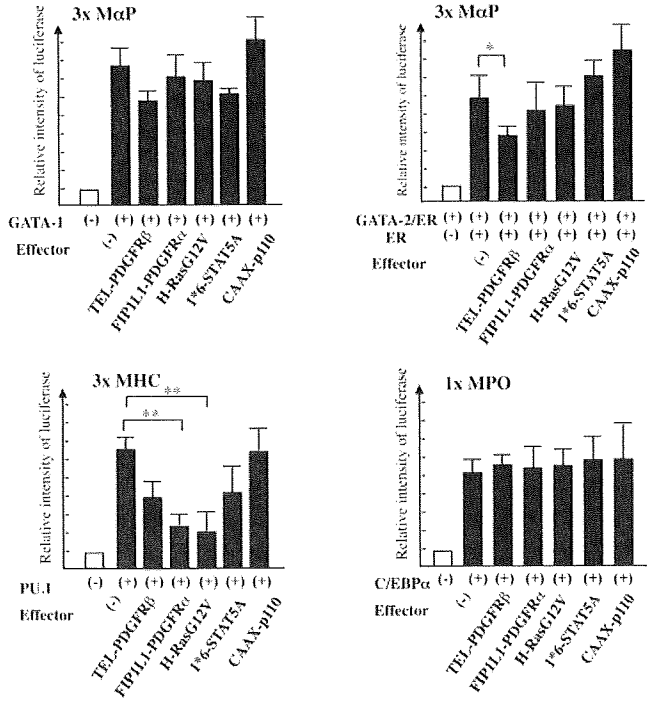


FIGURE 7. **Effects of FIP1L1-PDGFRα and its downstream molecules on the activities of eosinophil-related transcription factors.** The activities of GATA-1, GATA-2, PU.1, and C/EBPα were analyzed by luciferase assays. After transfection of several effector genes and the appropriate reporter genes, as indicated, NIH3T3 cells were cultured for 48 h and subjected to luciferase assays. 3×MαP-luciferase, 3×MHC-luciferase, and 1×MPO-luciferase contain biding sites for GATA, PU.1, and C/EBPα, respectively. *, $p < 0.05$; **, $p < 0.01$. Data represent means ± S.D. ($n = 3$).

Effects of FIP1L1-PDGFRα on the Expression and Activity of Lineage-specific Transcription Factors in KSLs—To further clarify the mechanism through which FIP1L1-PDGFRα enhanced eosinophil development, we analyzed the effects of FIP1L1-PDGFRα on the expression of *GATA-1*, *GATA-2*, *C/EBPα*, and *PU.1*, all of which have been reported to be key transcription factors for eosinophil development (45–47). To detect the changes in the expression of these factors that precede the phenotypic change, we isolated mRNA from sorted GFP-positive KSLs after 48-h retrovirus infection and performed semiquantitative RT-PCR analysis, since an apparent phenotypic change was not observed until 4 days (Fig. 2*A*, *top*). As shown in Fig. 6*A*, although the expression of *PU.1* was not so different among three transfectants, FIP1L1-PDGFRα augmented the expression of *C/EBPα* ($p < 0.01$) and *GATA-1* ($p <$

FIGURE 6. **Effects of FIP1L1-PDGFRα and its downstream molecules on the expressions of eosinophil-related transcription factors and effects of inhibition of these molecules.** *A*, the expressions of eosinophil-related transcription factors in KSLs were analyzed by RT-PCR analysis 48 h after retrovirus transfection. PCR products were electrophoresed and visualized by ethidium bromide staining (*left*), and their intensities were quantified using a Fluor Imager595 and ImageQuant software. Relative intensities to the products from mock-transduced cells are indicated (*right*). *, $p < 0.05$; **, $p < 0.01$ as compared with the value in mock-transduced cells. Data represent means ± S.D. ($n = 3$). *B*, murine KSLs were infected with lentivirus-expressing noncoding or encoding shRNA against C/EBPα or GATA-2 to evaluate the suppression efficacy of each shRNA. After a 48-h culture, cells were subjected to RT-PCR analyses (*top*). Next, FIP1L1-PDGFRα-transduced murine KSLs were further infected with these shRNAs and cultured with SCF, TPO, IL-6, and FLT3L, which were subjected to FACS analyses upon the expression of CD125 and Gr-1 (*bottom*). *, $p < 0.05$; **, $p < 0.01$ as compared with the value in the cells coexpressing FIP1L1-PDGFRα and noncoding shRNA ($n = 3$). *C*, FIP1L1-PDGFRα-transduced murine KSLs were further infected with retrovirus encoding mock or a dominant negative form of GATAs (GATA-3KRR). **, $p < 0.01$ as compared with the value in FIP1L1-PDGFRα- and mock-cotransduced cells ($n = 3$).

0.01) compared with mock-transduced KSLs. Furthermore, the expression of *GATA-2* was significantly higher in FIP1L1-PDGFRα-transduced KSLs than in mock- or TEL-PDGFRβ-transduced KSLs (FIP1L1-PDGFRα *versus* mock, $p < 0.01$).

To evaluate the roles for these transcription factors in FIP1L1-PDGFRα-induced eosinophil development, we inhibited the expression or function of these transcription factors using shRNAs or a dominant negative mutant. At first, we confirmed that these shRNAs suppressed the expression of *C/EBPα* and *GATA-2* considerably (Fig. 6*B*, *top*). When coexpressed with FIP1L1-PDGFRα in this condition, shRNA against C/EBPα reduced the FIP1L1-PDGFRα-induced $CD125^+Gr1^+$ fraction from 11.7 to 3.9% ($p < 0.01$) (Fig. 6*B*, *bottom*). Similarly, shRNA against GATA-2 suppressed this fraction to 6.8% ($p < 0.05$). Also, *GATA-3KRR*, which can inhibit both GATA-1 and GATA-2, reduced FIP1L1-PDGFRα-induced $CD125^+Gr1^+$ fraction from 13.9 to 3.5% ($p < 0.01$) (Fig. 6*C*). These results indicate that both GATA-2 and C/EBPα are required for FIP1L1-PDGFRα-induced eosinophil development.

We also examined the effects of FIP1L1-PDGFRα and its downstream signaling molecules (*i.e.* Ras, STAT5, and PI3-K) on transcription activities of these factors with luciferase assays using reporter genes and effector genes in combinations, as indicated in Fig. 7. In NIH3T3 cells, transiently transduced reporter genes for GATAs (3×*MαP*-luciferase), PU.1 (3×*MHC*-luciferase), and C/EBPα (1×*MPO*-luciferase) were activated by cotransfected GATA-1, PU.1, and C/EBPα by 7-fold, 7-fold, and 5-fold, respectively (Fig. 7). Also, the estradiol treatment activated 3×*MαP*-luciferase in GATA-2/ER-transfected cells. When FIP1L1-PDGFRα or a constitutively active form of H-Ras (H-RasG12V), STAT5 (1*6-STAT5A), or phosphatidylinositol 3-kinase (CAAX-p110) was further cotransfected, 1*6-STAT5A and CAAX-p110 scarcely affected transcription activities of GATA-1, GATA-2, PU.1, and C/EBPα. In contrast, both FIP1L1-PDGFRα and H-RasG12V reduced PU.1 activities to 30–40% ($p < 0.01$). Similar results were also obtained from 293T cells (data not shown). These results indicate that FIP1L1-PDGFRα regulates the expression and activities of various transcription factors, thereby promoting eosinophil development, and suggest that Ras may be a pivotal downstream mediator of FIP1L1-PDGFRα in this process.

DISCUSSION

In this study, we found that TEL-PDGFRα, but not FIP1L1-PDGFRβ, PDGFRαD562V, or PDGFRαD842V, promoted eosinophil development from KSLs as efficiently as FIP1L1-PDGFRα. This result indicates that constitutive TK activity transmitted from chimeric structure of PDGFRα is necessary to augment eosinophil development. In agreement with our finding, novel mutations identified in CEL were restricted to the chimeric form of PDGFRα (*i.e.* KIF5B-PDGFRα formed by t(4;10)(q12;p11), STRN-PDGFRα by t(2;4)(p24;q12), and ETV6-PDGFRα by t(4;12)(q2?3;p1?2)). As for the roles for downstream signaling molecules, the current results indicate that Ras/MEK and $p38^{MAPK}$ play essential roles in FIP1L1-PDGFRα-induced eosinophil development. However, this finding seems to be inconsistent with the fact that Ras/MEK is activated by various LTKs and normal hematopoietic growth factors. As for this reason, because FIP1L1-PDGFRα more intensely activated MEK/ERK and $p38^{MAPK}$ than TEL-PDGFRβ, we speculated that leukemogenic signals transmitted from chimeric PDGFRα would be quantitatively and qualitatively different from those from wild type TKs or other LTKs, thereby specifically promoting eosinophil development. In addition to the regulation of neoplastic cell proliferation, ERK has also been implicated in the control of signaling cascades associated with eosinophilia in asthma. Duan *et al.* (48) reported that an MEK inhibitor dramatically inhibited OVA-induced lung tissue eosinophilia and airway hyperresponsiveness. Also, $p38^{MAPK}$ is important for the induction of eosinophilia and function of terminal differentiated eosinophils in allergic airway inflammation (49, 50). In addition, our data suggest that $p38^{MAPK}$ would regulate eosinophil development at the early stage of hematopoiesis. Further studies to elucidate the crucial signal transduction mechanisms that control eosinophil development will provide a better rationale for the design of drug therapy not only for FIP1L1-PDGFRα-associated HES/CEL but also for allergic inflammation.

Our *in vitro* studies showed that FIP1L1-PDGFRα confers cytokine independence on KSLs and enhances their self-renewal activity, whereas it did not immortalize CMPs. In addition, although FIP1L1-PDGFRα-transduced KSLs caused MPD in recipient mice, FIP1L1-PDGFRα-transduced CMPs did not. These results indicate that FIP1L1-PDGFRα cannot confer self-renewal activity on CMPs and that the genetic alternation of FIP1L1-PDGFRα that causes CEL/HES occurs at an HSC level but not at a CMP level. In addition, we confirmed that mature eosinophils were generated from FIP1L1-PDGFRα-transduced KSLs in the presence of IL-5, indicating that FIP1L1-PDGFRα does not impair terminal differentiation of eosinophils. Also, when expressed in MEPs or CLPs, FIP1L1-PDGFRα brought about lineage conversion to eosinophil lineage. Together, these results suggest that, although LSCs harboring FIP1L1-PDGFRα derived from HSCs would continuously produce an excess number of mature eosinophils, a part of the eosinophils might be derived from FIP1L1-PDGFRα-harboring MEPs or CLPs.

In a previous report, FIP1L1-PDGFRα-transduced HSCs/HPCs caused myeloproliferative disorder in the recipient mice like BCR-ABL- or TEL-PDGFRβ-transduced KSLs (16, 51, 52), which was rather different from simple eosinophilia observed in human HES/CEL. Also in our transplantation experiment, none of the five mice transplanted with FIP1L1-PDGFRα-expressing KSLs developed eosinophilic disorders. However, we also observed that, whereas FIP1L1-PDGFRα-introduced KSLs differentiated up to $IL\text{-}5R\alpha^+$ eosinophil precursors under the cultures without IL-5, supplement of IL-5 let these $IL\text{-}5R\alpha^+$ cells undergo eosinophilic terminal differentiation. In accord with this hypothesis, Yamada *et al.* (52) reported that transplantation of FIP1L1-PDGFRα-transduced HSCs/HPCs obtained from IL-5 transgenic mice resulted in marked eosinophilia resembling HES/CEL in the recipient mice. Since p210BCR-ABL-transduced HSCs/HPCs did not cause eosinophilia even in the presence of IL-5 overexpression in the recipient mice, the induction of eosinophilia was attributable to FIP1L1-PDGFRα, Together with our results, these lines of evidence suggest that, although FIP1L1-PDGFRα is a major etiologic factor causing

eosinophilia, it is not sufficient to induce HES/CEL but requires additional events, such as IL-5 overexpression. In fact, some patients with FIP1L1-PDGFRα-associated HES were complicated with T-cell lymphoma (53–55). The frequency of FIP1L1-PDGFRα-induced HES/CEL was not as high (about 10%) as initially reported. However, similar LTK is supposed to be involved in the pathogenesis of HES/CEL, because imatinib is effective in some patients who do not have a *FIP1L1-PDGFRα* mutation (56). Also, a significant proportion of patients with HES/CEL have abnormal T-lymphocyte populations, such as $CD3^+CD4^-CD8^-$ and $CD3^-CD8^+$ T cells, which secret high levels of IL-5 (57). Currently, HES is categorized into two groups, "myeloproliferative variant" and "T-cell-mediated HES," and these groups are thought to be independent of each other (58, 59). However, because T-cell differentiation might be perturbed by FIP1L1-PDGFRα, it may be meaningful for the better understanding of the pathogenesis of HES/CEL to clarify the relationship between these two groups.

Iwasaki *et al.* (60) isolated eosinophil progenitors from murine BM, and they concluded that eosinophil developmental pathway would diverge from neutrophils and monocytes at the GMP stage. The lineage commitment of HSCs/HPCs and subsequent lineage-specific differentiation are crucially regulated by lineage-specific transcription factors, such as GATA-1, GATA-3, PU.1, C/EBPα, and C/EBPε. Among them, GATA-1 and PU.1 are known to antagonize each other and induce differentiation to erythroid/megakalyocyte or myeloid lineage, respectively (61–63). The CEBP family (CEBPα and CEBPε) is essential for the differentiation to myeloid lineage (64–66). FOG (Friend of GATA) and C/EBPβ regulate the eosinophil lineage induction antagonistically (67). Furthermore, enforced expression of C/EBPα converts MEPs to eosinophils (68), and expression of PU.1 converts them to GMPs (61, 67). Also, forced expression of GATA-1 in myeloid cells induces the formation of either MEPs or eosinophils, depending on the concentration of the factor (69). In addition, it was recently reported that C/EBPα expression followed by GATA-2 expression in GMPs is critical for eosinophil lineage specification (46). However, it is plausible that the mechanism of lineage commitment in leukemic cells is somewhat different from that in normal hematopoietic cells. In this study, we found that FIP1L1-PDGFRα enhanced the expression of GATA-1, GATA-2, and C/EBPα and suppressed PU.1 expression. Also, FIP1L1-PDGFRα suppressed transcription activities of PU.1. These results suggest that LTKs can influence the lineage commitment of HSCs/HPCs and subsequent differentiation by modifying the expression and activity of lineage-specific transcription factors.

In conclusion, we here found that FIP1L1-PDGFRα can enhance eosinophil development from HSCs/HPCs through the MEK/ERK and $p38^{MAPK}$ cascades by controlling the expression and activity of lineage-specific transcription factors. Furthermore, as far as we explored, this is the first report providing evidence that LTK has an ability to convert the lineage of committed progenitor cells. Further studies based on these findings would undoubtedly provide more useful information to understand the pathophysiology of various hematologic malignancies caused by LTKs.

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REFERENCES

1. Semerad, C. L., Poursine-Laurent, J., Liu, F., and Link, D. C. (1999) *Immunity* **11,** 153–161
2. Arcasoy, M. O., Maun, N. A., Perez, L., Forget, B. G., and Berliner, N. (2001) *Eur. J. Haematol.* **67,** 77–87
3. Hsu, C. L., Kikuchi, K., and Kondo, M. (2007) *Blood* **110,** 1420–1428
4. Buitenhuis, M., Verhagen, L. P., van Deutekom, H. W., Castor, A., Verploegen, S., Koenderman, L., Jacobsen, S. E., and Coffer, P. J. (2008) *Blood* **111,** 112–121
5. Radomska, H. S., Basseres, D. S., Zheng, R., Zhang, P., Dayaram, T., Yamamoto, Y., Sternberg, D. W., Lokker, N., Giese, N. A., Bohlander, S. K., Schnittger, S., Delmotte, M. H., Davis, R. J., Small, D., Hiddemann, W., Gilliland, D. G., and Tenen, D. G. (2006) *J. Exp. Med.* **203,** 371–381
6. Yamamoto, Y., Kiyoi, H., Nakano, Y., Suzuki, R., Kodera, Y., Miyawaki, S., Asou, N., Kuriyama, K., Yagasaki, F., Shimazaki, C., Akiyama, H., Saito, K., Nishimura, M., Motoji, T., Shinagawa, K., Takeshita, A., Saito, H., Ueda, R., Ohno, R., and Naoe, T. (2001) *Blood* **97,** 2434–2439
7. Levis, M., Tse, K. F., Smith, B. D., Garrett, E., and Small, D. (2001) *Blood* **98,** 885–887
8. Griffith, J., Black, J., Faerman, C., Swenson, L., Wynn, M., Lu, F., Lippke, J., and Saxena, K. (2004) *Mol. Cell.* **13,** 169–178
9. Bene, M. C., Bernier, M., Casasnovas, R. O., Castoldi, G., Knapp, W., Lanza, F., Ludwig, W. D., Matutes, E., Orfao, A., Sperling, C., and van't Veer, M. B. (1998) *Blood* **92,** 596–599
10. Nagata, H., Worobec, A. S., Oh, C. K., Chowdhury, B. A., Tannenbaum, S., Suzuki, Y., and Metcalfe, D. D. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 10560–10564
11. Longley, B. J., Tyrrell, L., Lu, S. Z., Ma, Y. S., Langley, K., Ding, T. G., Duffy, T., Jacobs, P., Tang, L. H., and Modlin, I. (1996) *Nat. Genet.* **12,** 312–314
12. Ikeda, H., Kanakura, Y., Tamaki, T., Kuriu, A., Kitayama, H., Ishikawa, J., Kanayama, Y., Yonezawa, T., Tarui, S., and Griffin, J. D. (1991) *Blood* **78,** 2962–2968
13. Longley, B. J., Jr., Metcalfe, D. D., Tharp, M., Wang, X., Tyrrell, L., Lu, S. Z., Heitjan, D., and Ma, Y. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 1609–1614
14. Fritsche-Polanz, R., Jordan, J. H., Feix, A., Sperr, W. R., Sunder-Plassmann, G., Valent, P., and Fodinger, M. (2001) *Br. J. Haematol.* **113,** 357–364
15. Furitsu, T., Tsujimura, T., Tono, T., Ikeda, H., Kitayama, H., Koshimizu, U., Sugahara, H., Butterfield, J. H., Ashman, L. K., Kanayama, Y., Matsuzawa, Y., Kitamura, Y., and Kanakura, Y. (1993) *J. Clin. Invest.* **92,** 1736–1744
16. Cools, J., Stover, E. H., Boulton, C. L., Gotlib, J., Legare, R. D., Amaral, S. M., Curley, D. P., Duclos, N., Rowan, R., Kutok, J. L., Lee, B. H., Williams, I. R., Coutre, S. E., Stone, R. M., DeAngelo, D. J., Marynen, P., Manley, P. W., Meyer, T., Fabbro, D., Neuberg, D., Weisberg, E., Griffin, J. D., and Gilliland, D. G. (2003) *Cancer Cell* **3,** 459–469
17. Klion, A. D., Noel, P., Akin, C., Law, M. A., Gilliland, D. G., Cools, J., Metcalfe, D. D., and Nutman, T. B. (2003) *Blood* **101,** 4660–4666
18. Stover, E. H., Chen, J., Lee, B. H., Cools, J., McDowell, E., Adelsperger, J., Cullen, D., Coburn, A., Moore, S. A., Okabe, R., Fabbro, D., Manley, P. W., Griffin, J. D., and Gilliland, D. G. (2005) *Blood* **106,** 3206–3213
19. Cools, J., DeAngelo, D. J., Gotlib, J., Stover, E. H., Legare, R. D., Cortes, J., Kutok, J., Clark, J., Galinsky, I., Griffin, J. D., Cross, N. C., Tefferi, A., Malone, J., Alam, R., Schrier, S. L., Schmid, J., Rose, M., Vandenberghe, P., Verhoef, G., Boogaerts, M., Wlodarska, I., Kantarjian, H., Marynen, P., Coutre, S. E., Stone, R., and Gilliland, D. G. (2003) *N. Engl. J. Med.* **348,** 1201–1214
20. Griffin, J. H., Leung, J., Bruner, R. J., Caligiuri, M. A., and Briesewitz, R. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 7830–7835
21. La Starza, R., Specchia, G., Cuneo, A., Beacci, D., Nozzoli, C., Luciano, L., Aventin, A., Sambani, C., Testoni, N., Foppoli, M., Invernizzi, R., Marynen, P., Martelli, M. F., and Mecucci, C. (2005) *Haematologica* **90,**

596–601
22. Buitenhuis, M., Verhagen, L. P., Cools, J., and Coffer, P. J. (2007) *Cancer Res.* **67,** 3759–3766
23. Bonnet, D., and Dick, J. E. (1997) *Nat. Med.* **3,** 730–737
24. Hope, K. J., Jin, L., and Dick, J. E. (2004) *Nat. Immunol.* **5,** 738–743
25. Sutherland, H. J., Blair, A., and Zapf, R. W. (1996) *Blood* **87,** 4754–4761
26. Blair, A., Hogge, D. E., and Sutherland, H. J. (1998) *Blood* **92,** 4325–4335
27. Cozzio, A., Passegue, E., Ayton, P. M., Karsunky, H., Cleary, M. L., and Weissman, I. L. (2003) *Genes Dev.* **17,** 3029–3035
28. Huntly, B. J., Shigematsu, H., Deguchi, K., Lee, B. H., Mizuno, S., Duclos, N., Rowan, R., Amaral, S., Curley, D., Williams, I. R., Akashi, K., and Gilliland, D. G. (2004) *Cancer Cell* **6,** 587–596
29. Akashi, K., Traver, D., Miyamoto, T., and Weissman, I. L. (2000) *Nature* **404,** 193–197
30. Segawa, K., Matsuda, M., Fukuhara, A., Morita, K., Okuno, Y., Komuro, R., and Shimomura, I. (2009) *J. Endocrinology* **200,** 107–116
31. Okitsu, Y., Takahashi, S., Minegishi, N., Kameoka, J., Kaku, M., Yamamoto, M., Sasaki, T., and Harigae, H. (2007) *Biochem. Biophys. Res. Commun.* **364,** 383–387
32. Smith, V. M., Lee, P. P., Szychowski, S., and Winoto, A. (1995) *The J. Biol. Chem.* **270,** 1515–1520
33. Ezoe, S., Matsumura, I., Nakata, S., Gale, K., Ishihara, K., Minegishi, N., Machii, T., Kitamura, T., Yamamoto, M., Enver, T., and Kanakura, Y. (2002) *Blood* **100,** 3512–3520
34. Ono, R., Ihara, M., Nakajima, H., Ozaki, K., Kataoka-Fujiwara, Y., Taki, T., Nagata, K., Inagaki, M., Yoshida, N., Kitamura, T., Hayashi, Y., Kinoshita, M., and Nosaka, T. (2005) *Mol. Cell. Biol.* **25,** 10965–10978
35. Ezoe, S., Matsumura, I., Gale, K., Satoh, Y., Ishikawa, J., Mizuki, M., Takahashi, S., Minegishi, N., Nakajima, K., Yamamoto, M., Enver, T., and Kanakura, Y. (2005) *J. Biol. Chem.* **280,** 13163–13170
36. Matsumura, I., Kawasaki, A., Tanaka, H., Sonoyama, J., Ezoe, S., Minegishi, N., Nakajima, K., Yamamoto, M., and Kanakura, Y. (2000) *Blood* **96,** 2440–2450
37. Matsumura, I., Kitamura, T., Wakao, H., Tanaka, H., Hashimoto, K., Albanese, C., Downward, J., Pestell, R. G., and Kanakura, Y. (1999) *EMBO J.* **18,** 1367–1377
38. Doornbos, R. P., Theelen, M., van der Hoeven, P. C., van Blitterswijk, W. J., Verkleij, A. J., and van Bergen en Henegouwen, P. M. (1999) *J. Biol. Chem.* **274,** 8589–8596
39. Tanaka, H., Matsumura, I., Itoh, K., Hatsuyama, A., Shikamura, M., Satoh, Y., Heike, T., Nakahata, T., and Kanakura, Y. (2006) *Stem Cells* **24,** 2592–2602
40. Abkowitz, J. L., Golinelli, D., Harrison, D. E., and Guttorp, P. (2000) *Blood* **96,** 3399–3405
41. Huang, S., Law, P., Francis, K., Palsson, B. O., and Ho, A. D. (1999) *Blood* **94,** 2595–2604
42. Stover, E. H., Chen, J., Folens, C., Lee, B. H., Mentens, N., Marynen, P., Williams, I. R., Gilliland, D. G., and Cools, J. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103,** 8078–8083
43. Heinrich, M. C., Corless, C. L., Duensing, A., McGreevey, L., Chen, C. J., Joseph, N., Singer, S., Griffith, D. J., Haley, A., Town, A., Demetri, G. D., Fletcher, C. D., and Fletcher, J. A. (2003) *Science* **299,** 708–710
44. Heldin, C. H., and Westermark, B. (1999) *Physiol. Rev.* **79,** 1283–1316
45. McNagny, K., and Graf, T. (2002) *J. Exp. Med.* **195,** F43–F47
46. Iwasaki, H., Mizuno, S., Arinobu, Y., Ozawa, H., Mori, Y., Shigematsu, H., Takatsu, K., Tenen, D. G., and Akashi, K. (2006) *Genes Dev.* **20,** 3010–3021
47. Du, J., Stankiewicz, M. J., Liu, Y., Xi, Q., Schmitz, J. E., Lekstrom-Himes, J. A., and Ackerman, S. J. (2002) *J. Biol. Chem.* **277,** 43481–43494
48. Duan, W., Chan, J. H., Wong, C. H., Leung, B. P., and Wong, W. S. (2004) *J. Immunol.* **172,** 7053–7059
49. Kampen, G. T., Stafford, S., Adachi, T., Jinquan, T., Quan, S., Grant, J. A., Skov, P. S., Poulsen, L. K., and Alam, R. (2000) *Blood* **95,** 1911–1917
50. Wong, C. K., Zhang, J. P., Ip, W. K., and Lam, C. W. (2002) *Clin. Exp. Immunol.* **128,** 483–489
51. Daley, G. Q., Van Etten, R. A., and Baltimore, D. (1990) *Science* **247,** 824–830
52. Yamada, Y., Rothenberg, M. E., Lee, A. W., Akei, H. S., Brandt, E. B., Williams, D. A., and Cancelas, J. A. (2006) *Blood* **107,** 4071–4079
53. McPherson, T., Cowen, E. W., McBurney, E., and Klion, A. D. (2006) *Br. J. Dermatol.* **155,** 824–826
54. Robyn, J., Lemery, S., McCoy, J. P., Kubofcik, J., Kim, Y. J., Pack, S., Nutman, T. B., Dunbar, C., and Klion, A. D. (2006) *Br. J. Haematol.* **132,** 286–292
55. Capovilla, M., Cayuela, J. M., Bilhou-Nabera, C., Gardin, C., Letestu, R., Baran-Marzak, F., Fenaux, P., and Martin, A. (2008) *Eur. J. Haematol.* **80,** 81–86
56. Jovanovic, J. V., Score, J., Waghorn, K., Cilloni, D., Gottardi, E., Metzgeroth, G., Erben, P., Popp, H., Walz, C., Hochhaus, A., Roche-Lestienne, C., Preudhomme, C., Solomon, E., Apperley, J., Rondoni, M., Ottaviani, E., Martinelli, G., Brito-Babapulle, F., Saglio, G., Hehlmann, R., Cross, N. C., Reiter, A., and Grimwade, D. (2007) *Blood* **109,** 4635–4640
57. Simon, H. U., Plotz, S. G., Dummer, R., and Blaser, K. (1999) *N. Engl. J. Med.* **341,** 1112–1120
58. Roufosse, F., Cogan, E., and Goldman, M. (2003) *Annu. Rev. Med.* **54,** 169–184
59. Roufosse, F. E., Goldman, M., and Cogan, E. (2003) *N. Engl. J. Med.* **348,** 2687; Author Reply 2687
60. Iwasaki, H., Mizuno, S., Mayfield, R., Shigematsu, H., Arinobu, Y., Seed, B., Gurish, M. F., Takatsu, K., and Akashi, K. (2005) *J. Exp. Med.* **201,** 1891–1897
61. Rekhtman, N., Radparvar, F., Evans, T., and Skoultchi, A. I. (1999) *Genes Dev.* **13,** 1398–1411
62. Zhang, P., Behre, G., Pan, J., Iwama, A., Wara-Aswapati, N., Radomska, H. S., Auron, P. E., Tenen, D. G., and Sun, Z. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 8705–8710
63. Nerlov, C., Querfurth, E., Kulessa, H., and Graf, T. (2000) *Blood* **95,** 2543–2551
64. Smith, L. T., Hohaus, S., Gonzalez, D. A., Dziennis, S. E., and Tenen, D. G. (1996) *Blood* **88,** 1234–1247
65. Hohaus, S., Petrovick, M. S., Voso, M. T., Sun, Z., Zhang, D. E., and Tenen, D. G. (1995) *Mol. Cell. Biol.* **15,** 5830–5845
66. Wang, N. D., Finegold, M. J., Bradley, A., Ou, C. N., Abdelsayed, S. V., Wilde, M. D., Taylor, L. R., Wilson, D. R., and Darlington, G. J. (1995) *Science* **269,** 1108–1112
67. Querfurth, E., Schuster, M., Kulessa, H., Crispino, J. D., Doderlein, G., Orkin, S. H., Graf, T., and Nerlov, C. (2000) *Genes Dev.* **14,** 2515–2525
68. McNagny, K. M., and Graf, T. (2003) *Blood* **101,** 1103–1110
69. Kulessa, H., Frampton, J., and Graf, T. (1995) *Genes Dev.* **9,** 1250–1262