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Role of the *KIT* protooncogene in normal and malignant human hematopoiesis

(leukemia/antisense DNA/transplantation)

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ABSTRACT **The role of the *KIT* protooncogene in human hematopoiesis is uncertain. Therefore, we examined KIT mRNA expression in normal human bone marrow mononuclear cells (MNC) and used antisense oligodeoxynucleotides (oligomers) to disrupt KIT function. KIT mRNA was detected with certainty only in growth factor-stimulated MNC. Expression was essentially abrogated by making MNC quiescent or by inhibiting *myb* gene function. Oligomers blocked KIT mRNA expression in a dose–response and sequence-specific manner, thereby allowing functional examination of the KIT receptor. In experiments with either partially purified or CD34$^+$-enriched MNC, neither granulocyte nor megakaryocyte colony formation was inhibited by oligomer exposure. In contrast, KIT antisense oligomers inhibited interleukin 3/erythropoietin-driven erythroid colony formation ≈70% and "stem cell factor"/erythropoietin-driven colony formation 100%. The presence of erythroid progenitor cell subsets with differential requirements for KIT function is therefore suggested. Growth of hematopoietic colonies from chronic myeloid leukemia and polycythemia vera patients was also inhibited, while acute leukemia colony growth appeared less sensitive to KIT deprivation. These results suggest that KIT plays a predominant role in normal erythropoiesis but may be important in regulating some types of malignant hematopoietic cell growth as well. They also suggest that KIT expression is linked to cell metabolic activity and that its expression may be regulated by or coregulated with MYB.**

The c-*kit* protooncogene is the normal cellular homologue of v-*kit*, the Hardy–Zuckerman 4 feline sarcoma virus oncogene (1). c-*kit* encodes a dimeric transmembrane tyrosine kinase receptor homologous to the colony-stimulating factor 1 and platelet-derived growth factor receptors (2). The mouse c-kit gene, *Kit*, has been mapped to chromosome 5, where it was determined to be allelic with the dominant white spotting locus (*W*) (3). *W* mice have a variety of abnormalities affecting coat color, gonadal development, and hematopoiesis. The latter is characterized by a marked reduction in the number of erythroid burst-forming units (BFU-E) (4) and a profound macrocytic anemia (5). The molecular lesions responsible for these defects are now being defined (6, 7).

The ligand for Kit has also recently been identified (reviewed in ref. 8). The encoded protein, known as stem cell factor (SCF), mast cell growth factor (MGF), or steel factor (SLF) is the product of a gene that resides at the steel (*Sl*) locus. Mice with *Sl* mutations have phenotypic abnormalities quite similar to those of *W* mice, and the explanation for this curiosity is now apparent. The *W* mouse lacks or has defects in a critical signal transducing receptor encoded by *Kit*, while the *Sl* mouse has defects in the ligand that stimulates the Kit receptor.

The above observations establish the critical importance of the Kit receptor in murine hematopoietic stem cell development, but its importance in human hematopoiesis remains unclear, since human mutations at the corresponding locus, *KIT*, on chromosome 4 have not been described. Therefore, to examine the role of the KIT receptor in human hematopoiesis, we examined KIT mRNA expression in normal human bone marrow mononuclear cells (MNC) and used antisense oligodeoxynucleotides (oligomers) to abrogate KIT function. Our results suggest that KIT plays a predominant role in normal erythropoiesis and that it might be of importance in regulating some types of malignant hematopoietic cell growth.

MATERIALS AND METHODS

Cells. Normal light-density MNC were obtained from consenting normal human donors and depleted of adherent cells and T lymphocytes (A^-T^- MNC) as described (9). In some experiments, CD34$^+$ cells were enriched from the A^-T^- MNC population by immunoselection with magnetic beads (Dynal, Oslo).

Malignant human hematopoietic cells were isolated by using the above techniques from the bone marrow or peripheral blood of patients with acute myelogenous leukemia (AML), acute lymphatic leukemia (ALL), Philadelphia chromosome-positive chronic myelogenous leukemia (CML), or polycythemia vera (PV).

Oligodeoxynucleotides. Unmodified, 18-nucleotide oligomers were synthesized and purified as reported (9–14). Oligomers were lyophilized to dryness and redissolved in culture medium prior to use [1 μg/μl (0.175 μM)]. Oligomer sequences used, corresponding to codons 1–6 of the published human KIT cDNA sequence (2), were as follows: 5′-ATG AGA GGC GCT CGC GGC-3′ sense oligomer, 5′-GCC GCG AGC GCC TCT CAT-3′ antisense oligomer, and 5′-GCA CCG TCT GCC AGT CGC-3′ scrambled-sequence oligomer.

Oligomer Exposure and Cell Culture. Cells were exposed to oligomers as described (14). A^-T^- MNC (2×10^5) or CD34$^+$ MNC (2×10^4) were incubated in 0.4 ml of Iscove's modified Dulbecco's medium (IMDM) containing 2% (vol/vol) human

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Abbreviations: MNC, bone marrow mononuclear cell(s); RT-PCR, reverse-transcription polymerase chain reaction; CFU, colony-forming unit(s); CFU-E, CFU-GM, CFU-MEG, erythroid, granulocyte/macrophage, and megakaryocyte CFU; BFU-E, erythroid burst-forming unit(s); SCF, stem cell factor; ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; PV, polycythemia vera; EPO, erythropoietin.

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AB serum and 10 mM Hepes buffer in polypropylene tubes (Fisher Scientific). Oligomers (2.5–100 μg/ml) were added at time zero, and 50% of the initial dose was added again 18 hr later (final total concentration, ≈0.6–26 μM). Twenty-four hours after the first addition of oligomers, cells were prepared for plating in plasma clot or methylcellulose cultures as reported (9, 13, 14). Cells (1×10^5 A^-T^- MNC or 1×10^4 $CD34^+$ MNC per dish) were not washed before plating. Control cultures were manipulated in an identical manner but did not contain oligomers.

Assays for hematopoietic progenitor cells of various lineages were carried out as reported (9, 13, 14). Erythroid colony-forming units (CFU-E) were stimulated with erythropoietin (EPO; 5 units/ml); BFU-E, with EPO and interleukin 3 (IL-3; 20 units/ml) or EPO and SCF (100 ng/ml); granulocyte/macrophage colony-forming units (CFU-GM), with IL-3 (20 units/ml) and granulocyte/macrophage colony-stimulating activity (5 ng/ml)]; and megakaryocyte colony-forming units (CFU-MEG), with IL-3 (20 units/ml) and IL-6 (100 ng/ml). Colony identification was carried out as described (9, 13, 14).

Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Total RNA was extracted from cells ($2–5 \times 10^6$) as reported (14). RNA was reverse-transcribed with 500 units of Moloney murine leukemia virus reverse transcriptase (Mo-MLV-RT) and 50 pmol of a 22-nucleotide 3′ primer complementary to nucleotides 1201–1179 [CTA GGA ATG TGT AAG TGC CTC C] of the published KIT cDNA sequence (2). The resulting cDNA fragment was amplified by using 5 units of *Thermus aquaticus* (*Taq*) polymerase and a 22-nucleotide 5′ primer specific for nucleotides 842–864 [CGT TGA CTA TCA GTT CAG CGA G]. Twenty-five microliters of amplified product was electrophoresed on 4% agarose gel and subsequently transferred to a nylon filter. Filters were prehybridized and then probed with a ^{32}P-end-labeled oligonucleotide probe corresponding to a 21-nucleotide KIT sequence [GAT CCA CTG CTG GTG TTC AGG] contained within the amplified region (nucleotides 1068–1047). Autoradiography was performed by exposing filters on Kodak x-ray film at −70°C with intensifying screens.

Statistical Analysis. Statistical significance of differences in numbers of colonies arising in the duplicate control, sense, or antisense plates was determined by using the Student *t* test for unpaired samples. *P* values less than 0.05 were judged to be of statistical significance.

RESULTS

Expression of KIT mRNA in Normal MNC. We first sought to determine (*i*) if expression of KIT mRNA was detectable in MNC, (*ii*) the relationship between *KIT* gene expression and cellular metabolic activity, and (*iii*) if KIT mRNA expression varied with time in culture. To carry out these studies, A^-T^- MNC were placed in tissue culture medium containing 2% normal human AB serum at 4°C. Twenty-four hours later, the cells were rapidly warmed to 37°C, the culture medium serum concentration was adjusted to 5% (vol/vol) and supplemented with 20 units of recombinant human IL-3 and 5 units of erythropoietin per ml. Immediately (time 0) and at 2-, 8-, 12-, 24-, 36-, and 48-hr intervals thereafter, RNA was extracted from cells for RT-PCR-based analysis of KIT expression (Fig. 1). MNC at 4°C appeared to express KIT mRNA at extremely low levels, since a signal was detectable at time 0 only after prolonged exposure of the autoradiogram. Within 2 hr of stimulation, however, KIT expression was unequivocally detectable. It continued to increase through 24 hr, at which time expression appeared to peak and persisted through 48 hr. Though PCR is at best semiquantitative, the fact that equivalent results were obtained on three separate occasions suggests that these results are likely to reflect real-time kinetics.

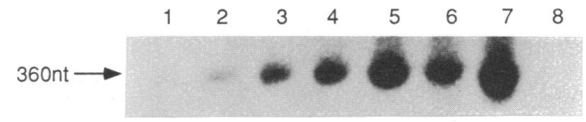

FIG. 1. Expression of KIT mRNA in A^-T^- MNC. A^-T^- MNC were cultured for 24 hr at 4°C and then rewarmed to 37°C in the presence of growth factors as described in the text. Immediately (lane 1) and at 2-, 8-, 12-, 24-, 36-, and 48-hr intervals thereafter (lanes 2–7, respectively), total cellular RNA was extracted for RT-PCR-based analysis of KIT expression. A simultaneous control reaction containing the PCR mixture without RNA is shown in lane 8. nt, Nucleotides.

The results of these experiments suggested the possibility that *KIT* expression might also be linked to cell cycle activity. To explore this further, we examined the relationship between *MYB* expression, a critical regulator of G_1/S transition (11), and *KIT* expression (Fig. 2). MNC were exposed to *MYB* antisense oligomers and then cultured as described in the legend of Fig. 2. We again found KIT mRNA to be weakly expressed immediately after rewarming from 4°C (Fig. 2 *Upper*, lane 1) but detectable in control and MYB sense-oligomer-treated cells after 36 hr (37°C in the presence of growth factors) (Fig. 2 *Upper*, lanes 2 and 3). In marked contrast, KIT mRNA was not detectable in cells exposed to the MYB antisense oligomers (Fig. 2 *Upper*, lane 4). Simultaneous assay for β-actin message showed no significant change in mRNA levels, suggesting that the decrement in *KIT* expression was specifically related to down-regulation of *MYB* (Fig. 2 *Lower*). These results suggest that in normal hematopoietic cells, *KIT* may be regulated by or coregulated with *MYB*.

Effect of KIT Oligomers on Normal Human Hematopoietic Progenitor Cell Growth. We then determined if *KIT* expression in MNC could be abrogated in a sequence-specific manner by exposure to KIT antisense oligodeoxynucleotides (Fig. 3). A^-T^- MNC (2×10^6) were again incubated at 4°C for 24 hr in a low serum (2%)-containing medium. Cells were rewarmed to 37°C and exposed to growth factors as described above in the presence of KIT oligomers. After 36 hr, total RNA was extracted for RT-PCR detection of KIT message. KIT mRNA was expressed at a very low level after 24 hr at 4°C (Fig. 3). Thirty six hours after warming, KIT mRNA was detected in control cells and in cells exposed to sense and

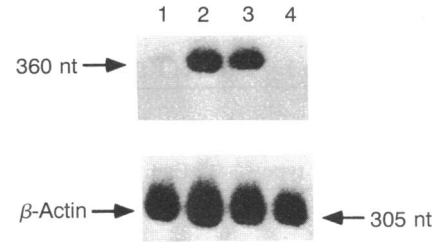

FIG. 2. Effect of MYB oligodeoxynucleotides on KIT expression in human marrow mononuclear cells. A^-T^- MNC (5×10^6) were placed at 4°C for 24 hr in 5 ml polypropylene tubes (Fisher Scientific) in a total volume of 0.4 ml of Iscove's modified Dulbecco's medium containing 2% human AB serum and 10 mM Hepes buffer. Cells were then rewarmed to 37°C in supplemented α medium containing 5% AB serum, IL-3 (20 units/ml) and EPO (5 units/ml). Oligomers were added at the time of rewarming (time 0; 80 μg/ml), and 50% of the initial dose was added again 18 hr later. Thirty-six hours after the first addition of oligomers, total RNA was extracted for RT-PCR analysis of KIT expression (*Upper*) and β-actin expression (*Lower*) (10). (*Upper*) Lanes: 1 time 0 (control); 2, 36-hr control; 3, cells exposed to MYB sense oligomers; 4, cells exposed to MYB antisense oligomers. (*Lower*) Lanes: 1, time 0 (control); 2, 36-hr control; 3, cells exposed to MYB sense oligomers; 4, cells exposed to MYB antisense oligomers. nt, Nucleotides.

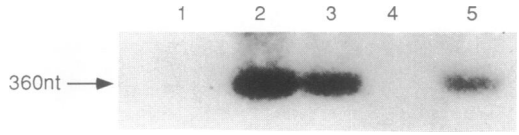

FIG. 3. Expression of KIT mRNAs in normal MNC exposed to KIT oligomers. A^-T^- MNC were cultured as described in the legend of Fig. 1. Cells were exposed to oligomers as previously described (6). A^-T^- MNC (2×10^6) were incubated as described in Fig. 1, oligomers were added at time zero (100 μg/ml), and 50% of the initial dose was added again 18 hr later. At time 0 and 36 hr after the first addition of oligomers, total RNA was extracted for RT-PCR analysis of KIT expression (10). Lanes: 1, time 0 (control); 2, 36-hr control; 3, cells exposed to KIT sense oligomers; 4, cells exposed to KIT antisense oligomers; 5, cells exposed to scramble-sequence KIT oligomers. nt, Nucleotides.

scrambled-sequence oligomers. In contrast, antisense-treated cells had no detectable KIT mRNA. Accordingly, inhibition of *KIT* expression was highly efficient and sequence specific.

The effect of KIT antisense and control sequence oligomers on normal hematopoietic progenitor cell cloning efficiency and development was then systematically investigated by assessing the effect of oligomer exposure on CFU-E-, BFU-E-, CFU-GM-, and CFU-MEG-derived colony growth. KIT antisense oligomers inhibited erythroid colony formation in a dose-dependent fashion (Fig. 4). Inhibition was sequence specific since neither sense nor scrambled-sequence oligomers significantly affected colony growth in comparison with untreated controls. At the highest doses used, nonspecific inhibition of erythroid colony formation was sometimes seen (experiment 2 in Table 1), but this inhibition was never as great as that observed with the antisense oligomers. These inhibited growth of CFU-E and BFU-E ≈75% and ≈71%, respectively (Table 1). Residual colonies were much smaller than those of the untreated controls (Fig. 5). Nevertheless, maturation of the cellular constituents of the colonies appeared to be normal in terms of the cells' ability to synthesize hemoglobin (Fig. 5). Indirect immunofluorescence analysis of cell hemoglobin content using antibodies recognizing hemoglobins F and A (Calbiochem) revealed no discernible difference in the type or percentage of hemoglobin synthesized by the antisense-treated cells (data not shown). In contrast to the erythroid colony results, CFU-GM- and CFU-MEG-derived colony

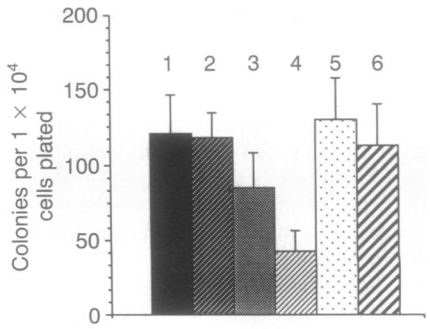

FIG. 4. Effect of KIT oligodeoxynucleotides on BFU-E-derived colony formation. $CD34^+$ MNC (1×10^4) were incubated as described in Fig. 1, oligomers were added at time 0 (2.5–100 μg/ml), and 50% of the initial dose was added again 18 hr later (final total concentration ≈ 1–52.5 μM). Twenty-four hours after the first addition of oligomers, cells were prepared for plating in plasma clot or methylcellulose cultures (6, 7). Cells were not washed before plating. Control cell cultures were untreated. Bars: 1, Untreated control cells; 2, Antisense treatment of 20 μg/ml followed by 10 μg/ml; 3, antisense treatment of 40 μg/ml followed by 20 μg/ml; 4, antisense treatment of 100 μg/ml followed by 50 μg/ml; 5, sense treatment of 100 μg/ml followed by 50 μg/ml; 6, scrambled-sequence treatment of 100 μg/ml followed by 50 μg/ml.

formation was unaffected by exposure to any of the oligomers at any of the doses used (Table 1).

Similar results were obtained after exposure of $CD34^+$ cells to KIT antisense oligomers. The mean number of BFU-E colonies decreased ≈71% (Table 2). Neither sense nor scrambled-sequence oligomers significantly inhibited erythroid colony formation. As was also noted with the less pure A^-T^- population, neither antisense nor control oligomers inhibited CFU-GM colony formation.

To provide additional proof that KIT antisense-mediated inhibition of erythropoiesis was due to the absence of KIT receptor function, we sought to demonstrate that BFU-E responsiveness to SCF could be abolished in a sequence-specific manner after exposure to KIT oligomers. Accordingly $CD34^+$ MNC (1×10^4) were cloned in the presence of 5 units of EPO and 100 ng of SCF per ml alone or with sense, antisense, or scrambled-sequence KIT oligomers [final concentration, 150 μg/ml (≈26 μM)]. In four experiments, 191 ± 19 BFU-E (mean ± SD) were grown in the presence of the growth factors alone. These numbers were not statistically different from those cloned with sense (183 ± 29; $P = 0.654$)

Table 1. Effect of KIT oligomers on A^-T^- MNC colony formation

A^-T^- cell type	Exp.	Effect on progenitor cell growth, no. of colonies			
		Control	Sense oligomer	Scrambled sequence	Antisense oligomer
CFU-E	1	182, 209	153, 142	119, 128	33, 59
	2	1943, 543	1635, 1135	627, 649	243, 213
	3	148, 110	129, 176	149, 206	97, 107
	Mean ± SEM	522 ± 291	562 ± 268	313 ± 103	125 ± 34
BFU-E	1	133, 152	117, 106	94, 64	60, 149
	2	534, 392	601, 249	273, 246	126, 113
	3	206, 172	215, 258	162, 246	59, 51
	Mean ± SEM	265 ± 66	258 ± 74	181 ± 36	76 ± 14
CFU-GM	1	212, 189	231, 179	282, 193	195, 220
	2	412, 408	395, 421	457, 384	407, 471
	3	217, 241, 209, 246	230, 237	201, 199	293, 187
	Mean ± SEM	280 ± 42	282 ± 41	286 ± 46	296 ± 49
CFU-MEG	1	114, 107	133, 117	154, 113	127, 112
	2	93, 100	58, 52	53, 40	47, 54
	Mean ± SEM	104 ± 5	90 ± 20	90 ± 27	85 ± 20

A^-T^- MNC (2×10^5) were incubated and treated as described in *Materials and Methods*. Values are actual colony counts from two or three individual studies at the highest concentration tested.

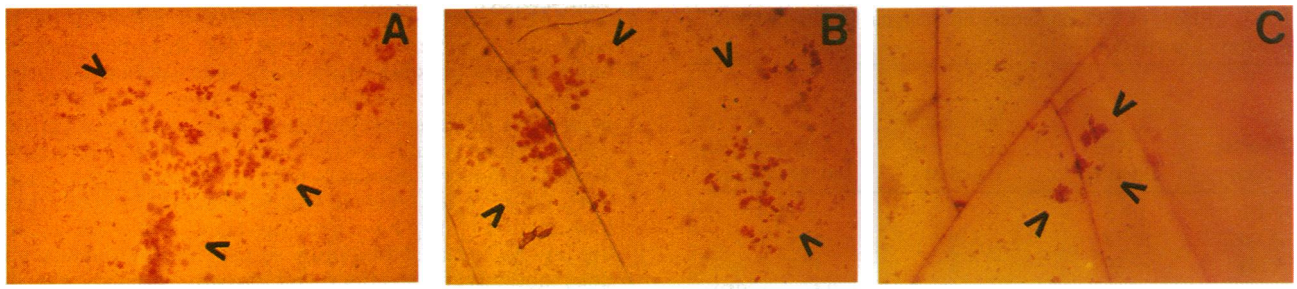

FIG. 5. Human BFU-E cloned in plasma clot culture as detailed in the legend of Fig. 4. (*A*) Untreated control cell colony. (*B*) Colony derived from cells exposed to KIT sense oligomers. (*C*) Colony derived from cells exposed to KIT antisense oligomers. Colonies are delineated by open arrow heads. (×95.)

or scrambled-sequence oligomers (180 ± 20; $P = 0.758$). In the presence of the kit antisense oligomers, BFU-E-derived colony formation was completely abolished (0.4 ± 0.7; $P < 0.0001$), suggesting that KIT receptor was no longer present to interact with its ligand.

Role of KIT Receptor in Malignant Hematopoietic Cell Growth. The above data suggest that the *KIT*-encoded receptor plays an important role in normal hematopoiesis, especially for erythroid cell development. KIT mRNA and protein has been detected in some acute leukemias, suggesting a role for the *KIT*-encoded protein in malignant hematopoietic cell growth as well (15, 16). Nevertheless, its importance in the pathogenesis of these diseases remains unclear, since expression in primary leukemias is quite variable (15, 16) and, to our knowledge, has not been examined in other malignant hematopoietic diseases such as the myeloproliferative disorders. To address this issue, A^-T^- MNC were obtained from patients with a variety of hematologic malignancies and were exposed to the KIT oligomers. The effect of oligomer exposure on the ability of malignant CFU-GM to form colonies in semisolid medium was then assessed (Table 3). Twenty-one patients were studied: 3 with ALL, 4 with AML, 10 with CML, and 4 with PV. CFU-GM from 11 of these patients were inhibited by exposure to the KIT antisense oligomers. Of these, 9 were derived from patients with myeloproliferative disorders (all but one indeterminate case had PCR-documented expression of KIT mRNA; data not shown), suggesting that progenitor cells isolated from patients with these disorders were more dependent on KIT function than were those with acute leukemia. Five responding patients had CML. Interestingly, when residual colonies were probed for bcr-abl-expressing cells by RT-PCR (14, 17), bcr-abl expression was substantially decreased or undetectable in cases where colony inhibition was observed. In patients with PV, both BFU-E- (92% ± 3) and CFU-GM-derived colony formation (Table 3) were significantly inhibited by the KIT antisense oligomers.

DISCUSSION

The importance of the KIT receptor in murine hematopoiesis is undisputed. Inferential data that the receptor plays a significant role in human hematopoiesis are also accumulating, primarily in the form of studies demonstrating that the KIT ligand stimulates the growth of hematopoietic colonies (8, 18). The KIT ligand appears to have little colony-stimulating activity on its own but instead acts synergistically with other growth factors, in particular with IL-3, to enhance colony formation. Nevertheless, because a certain amount of "cross talk" is known to exist among the hematopoietic growth factors, the importance of the KIT ligand's receptor in regulating hematopoietic cell development remains undefined. Similarly, the biological function(s) governed by the KIT receptor remain unspecified. Given its similarity to other growth factor receptors, it is likely a component of a growth factor signal-transduction apparatus. Other functions, such as serving as a cell surface adhesion molecule receptor, are also possible (19).

Though the exact function of the receptor remains undefined, the data presented herein provide insight into factors that regulate *KIT* expression in normal human MNC. In this regard, we found (*i*) that expression of KIT was virtually undetectable in cells that had been rendered quiescent by exposure to cold and a low serum environment and (*ii*) that expression increased after cells were rewarmed and then stimulated with IL-3 and erythropoietin (Fig. 1). Whether the augmented KIT expression we found was due to increased metabolic activity of the cell or was related to initiation of cell cycle activity remains to be determined. Nevertheless, since

Table 2. Effect of kit oligomers on $CD34^+$ colony formation

$CD34^+$ cell type	Exp.	Effect on progenitor cell growth, no. of colonies			
		Control	Sense oligomer	Scrambled sequence	Antisense oligomer
BFU-E	1	179, 229	293, 120	191, 261	81, 75
	2	276, 281	241, 151	227, 283	34, 91
	3	271, 451	440, 361	321, 351	92, 111
	Mean ± SEM	281 ± 37	268 ± 50	272 ± 24	81 ± 11
CFU-GM	1	321, 299	287, 360	321, 339	354, 319
	2	309, 312	316, 262	289, 324	349, 271
	3	114, 121, 90, 135	109, 103	84, 106	94, 103
	Mean ± SEM	213 ± 37	240 ± 44	244 ± 48	248 ± 49

A^-T^- MNC cells were suspended in supplemented α medium and incubated with mouse anti-HPCA-I antibody in 1:20 dilution for 45 min at 4°C with gentle inverting of tubes. Cells were washed three times in supplemented α medium and then incubated with beads coated with the Fc fragment of goat anti-mouse IgG_1 (75 μl of ImmunoBeads per 10^7 A^-T^- MNC). After 45 min of incubation at 4°C, cells adherent to the beads were positively selected by using a magnetic particle concentrator. $CD34^+$ MNC (2×10^4) were exposed to oligomers as described in *Materials and Methods*. Values are actual colony counts from three or four individual studies at the highest oligomer concentration tested.

Table 3. Effect of KIT oligomers on malignant hematopoietic cell colony growth (CFU-GM)

Disease type	Plates: No. studied	Plates: No. with decrease in colonies	% decrease in no. of colonies
ALL	3	1	68%
AML	4	1	63%
CML	10	5	65% ± 26% (Mean ± SD)
PV	4	4	74% ± 24 (Mean ± SD)
Total = 21		11	Avg = 68%

Cells were exposed to oligomers and cultured as described in *Materials and Methods*.

KIT expression appeared relatively early after stimulation (≈2 hr, Fig. 2), initial expression in G_0 cells would not appear to be dependent on the activation of "late" cell cycle genes such as *MYB*. The role of early genes, such as *MYC*, in triggering *KIT* expression needs exploration. However, since pertubation of MYB function led to downregulation of *KIT* expression at 36 hr without affecting β-actin mRNA expression, *KIT* may be regulated by different mechanisms in cells that are cycling and in a manner independent of cellular metabolic activity. These regulatory mechanisms also require elucidation but may be MYB-related, at least in normal cells.

These findings conflict with a recent report suggesting that *KIT* expression is unrelated to cellular metabolic activity or cell cycle state and that mRNA levels decline after cells are exposed to hematopoietic growth factors (20). Different target cells may be one explanation for these discordant results. On the other hand, our data are consistent with the observation that KIT ligand synergizes with IL-3-stimulated cells in promoting erythroid colony growth (4). These widely observed results are difficult to reconcile with the observation that exposure to IL-3 might down-regulate KIT receptor mRNA and protein (20).

In addition to elucidating biological factors that regulate *KIT* expression, the experimental approach used has also generated some unexpected findings about the importance of *KIT* expression in human hematopoietic cell development. First, the KIT receptor does not appear to play a critical role in either myeloid or megakaryocyte development, at least at the early progenitor cell level. This statement follows from the observation that cells of both lineages appear to develop normally after exposure to the antisense oligomers. Whether development from even earlier progenitor cells than those assayed or from cells with more "stem"-like properties would also be unaffected is unclear. In marked contrast to these results, however, erythroid colony development at both the BFU-E and CFU-E level appeared to be markedly dependent on KIT function. This dependence appeared to be most critical during the early events of colony formation, since maturation in residual colonies was otherwise normal. Second, the results also strongly imply the existence of two distinct subsets of erythroid progenitors with different dependence on the KIT ligand, and therefore the KIT receptor. These subsets may differ in their maturational state and other as yet unidentified properties.

Finally, the KIT receptor may be of importance in regulating growth of some malignant hematopoietic cell types as well. In this regard, it was found that growth of granulocyte colony-forming units from most cases of acute leukemia, either AML or ALL, was not inhibited by exposure to the KIT antisense oligomers. In contrast, growth of granulocyte colony-forming units derived from patients with CML and PV was much more sensitive to the inhibitory effects of the KIT antisense oligomers. The reason for this discrepancy is unclear. One possible explanation may be that CFU from patients with acute leukemia are blocked at a maturation level where KIT function is relatively unimportant for cell growth. This situation would contrast with CFU from patients with myeloproliferative disorders who are likely to have progenitor cells at varied levels of maturation. Alternatively, acute leukemia CFU may have generated alternate ways of carrying out KIT-related functions so that receptor deprivation does not adversely effect cell growth. Finally, the data suggest that KIT may subserve different functions in normal as opposed to malignant hematopoietic cells since CFU-GM-derived granulocyte–macrophage colony formation was unaffected by KIT disruption in the former but led to inhibition of colony formation in the latter cell types. It is possible that these differences can be exploited for therapeutic purposes.

Noted Added in Proof. While this manuscript was under review, Giebel and Spritz, and Fleischman *et al.* reported, respectively, that mutation (21) and heterozygous deletion (22) of *KIT* could result in human piebaldism. Mutations associated with abnormal human hematopoiesis have yet to be described.

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