Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 459–463, January 1994
Cell Biology

STK-1, the human homolog of Flk-2/Flt-3, is selectively expressed in CD34+ human bone marrow cells and is involved in the proliferation of early progenitor/stem cells

(growth factor receptor/tyrosine kinase/hematopoiesis/antisense)

DONALD SMALL*†‡, MARK LEVENSTEIN*, EUNKYUNG KIM*, CATHY CAROW*, SHAHINA AMIN*, PATRICIA ROCKWELL§, LARRY WITTE§, CHRISTOPHER BURROW¶, MARIUSZ Z. RATAJCZAK‖, ALAN M. GEWIRTZ‖**, AND CURT I. CIVIN*†

*Oncology Center, Departments of †Pediatrics and ¶Nephrology, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, MD 21287; Departments of ‖Laboratory Medicine and **Pathology, Department of Medicine, University of Pennsylvania School of Medicine, 36th Street and Hamilton Walk, Philadelphia, PA 19104; and §ImClone Systems, Inc., 180 Varick Street, New York, NY 10014

Communicated by Bert Vogelstein, September 28, 1993

ABSTRACT **We cloned the cDNA for stem cell tyrosine kinase 1 (STK-1), the human homolog of murine Flk-2/Flt-3, from a CD34+ hematopoietic stem cell-enriched library and investigated its expression in subsets of normal human bone marrow. The cDNA encodes a protein of 993 aa with 85% identity and 92% similarity to Flk-2/Flt-3. STK-1 is a member of the type III receptor tyrosine kinase family that includes KIT (steel factor receptor), FMS (colony-stimulating factor 1R), and platelet-derived growth factor receptor. STK-1 expression in human blood and marrow is restricted to CD34+ cells, a population greatly enriched for stem/progenitor cells. Anti-STK-1 antiserum recognizes polypeptides of 160 and 130 kDa in several STK-1-expressing cell lines and in 3T3 cells transfected with a STK-1 expression vector. Antisense oligonucleotides directed against STK-1 sequences inhibited hematopoietic colony formation, most strongly in long-term bone marrow cultures. These data suggest that STK-1 may function as a growth factor receptor on hematopoietic stem and/or progenitor cells.**

Lymphohematopoietic stem cells (LHSCs) serve as the reservoir for virtually all blood cells but make up only ≈0.01% of human/murine marrow cells (1, 2). The ability to isolate and expand this population would facilitate the study of early hematopoietic development and has clinical applications in bone marrow transplantation for cancer and genetic diseases. Immunoaffinity-purified CD34+ cells contain virtually all of the progenitor activity of marrow and include LHSCs (3–7).

Cytokines bind to transmembrane receptors, which often have endogenous tyrosine kinase (TK) activity within their cytoplasmic domain (8). Receptor TKs can be grouped into the epidermal, fibroblast, and platelet-derived growth factor receptors, and insulin receptor families (9–12). Several receptor TKs have been shown to be important in the differentiation and proliferation of hematopoietic cells. FMS is important for growth and differentiation of the monocyte/macrophage/osteoclast lineage, and KIT plays a role in similar processes in early hematopoietic cells as well as mast cells, melanocytes, and germ cells (13–15). As a step in a strategy to isolate and clone growth factors for human LHSCs, we attempted to clone receptor TK cDNAs specific to the CD34+ fraction of human bone marrow.

The cloning strategy we used takes advantage of the highly conserved catalytic domain of TKs (16). We cloned the cDNA for stem cell TK-1 (STK-1), the human homolog of murine Flk-2/Flt-3, and demonstrated its selective expression in normal human CD34+ marrow cells. Antisense (AS) inhibition experiments suggest that STK-1 plays an essential role in regulation of hematopoiesis.††

MATERIALS AND METHODS

Isolation of Bone Marrow and Peripheral Blood Cells. Normal iliac crest bone marrow was aspirated from consenting healthy human volunteers under an Institutional Review Board approved protocol. Cell subsets were purified by immunomagnetic separation (17, 18).

Cloning of STK-1. RNA was isolated by the guanidium thiocyanate method (19). First-strand cDNA, generated from 1 μg of CD34+ total RNA with priming by random hexamers and reverse transcription by Moloney murine leukemia virus reverse transcriptase (M-MLV-RT) (GIBCO/BRL), was used as template. Degenerate oligonucleotide primers (designated HHSC-PTK1 and HHSC-PTK2), corresponding to the consensus sequences of the VIb and IX subdomains of TKs, were used for PCR amplification (11). A DNA fragment (HHSC-PTK.2A) was isolated with predicted amino acid identity of 97% when compared to the murine Flk-2/Flt-3. A CD34+ cDNA library was constructed using the Superscript plasmid system (GIBCO/BRL) and screened with this fragment. Positive clones were isolated and after sequencing (20) 5′ rapid amplification of cDNA ends (21, 22) was used to complete the missing 5′ end of the cDNA.

Reverse Transcriptase PCR (RT-PCR). Equal amounts (usually 1 μg) of total RNA were reverse transcribed with M-MLV-RT using random hexamers or oligo(dt)$_{15}$ (Boehringer Mannheim) as primers. Aliquots of this material were used with specific primer pairs for PCR amplification. Primer pairs used for STK-1 amplification included nt 91–117 and 324–304 or 878–895 and 1557–1540. Amplification consisted of 95°C for 5 min before adding *Taq* polymerase (New England Biolabs), followed by cycles of 95°C for 1 min, 45°C for 1 min, and 72°C for 2 min, repeated for 35 cycles. The PCR products were electrophoresed through 1% agarose gels in TAE buffer and transferred overnight to nitrocellulose (23), processed, and probed (24).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LHSC, lymphohematopoietic stem cell; STK-1, stem cell tyrosine kinase 1; TK, tyrosine kinase; LTBMC, long-term bone marrow culture; ODN, oligonucleotide; CFU, colony-forming unit; GM, granulocyte/macrophage; GEMM, granulocyte/erythrocyte/monocyte/megakaryocyte; RT-PCR, reverse transcriptase PCR; US, upstream; TS, translation start site; DS, downstream; S, sense; AS, antisense; Scr, scrambled.

‡To whom reprint requests should be addressed.

††The sequence reported in this paper has been deposited in the GenBank data base (accession no. U02687).

RNase Protection. A 5′ fragment of STK-1 extending from nt 70–269 was cloned into pBluescript II KS− downstream of the T3 promoter such that transcription from the T3 promoter produced a 330-nt transcript with 130 nt of vector sequence followed by 200 nt of AS STK-1. The assay was performed as described (25).

Western Blotting. Equal amounts of protein were electrophoresed and transferred to nitrocellulose filters (26). Filters were incubated for 2 hr at room temperature with affinity-purified rabbit polyclonal antibody (5 μg/ml) raised against a peptide from the kinase insert region (aa 740–757). The peptide blocking experiment was performed with 20 μg of the immunogenic peptide per ml added to the antibody for 1 hr at room temperature before incubation with the filter. After washing, the filters were incubated with horseradish peroxidase-conjugated secondary antibody (mouse anti-rabbit) at 1:1500 and detected by ECL (Amersham).

AS Inhibition Experiments. Unmodified 18-base oligodeoxynucleotides (ODNs) were synthesized and purified (27–29). Three AS oligomers were synthesized based on the STK-1 cDNA sequence to target to nt 1–18 (upstream; US), 58–75 (translational start site; TS), and 121–138 (downstream; DS). All experiments were carried out with corresponding sense (S) sequence and scrambled (Scr) sequence controls.

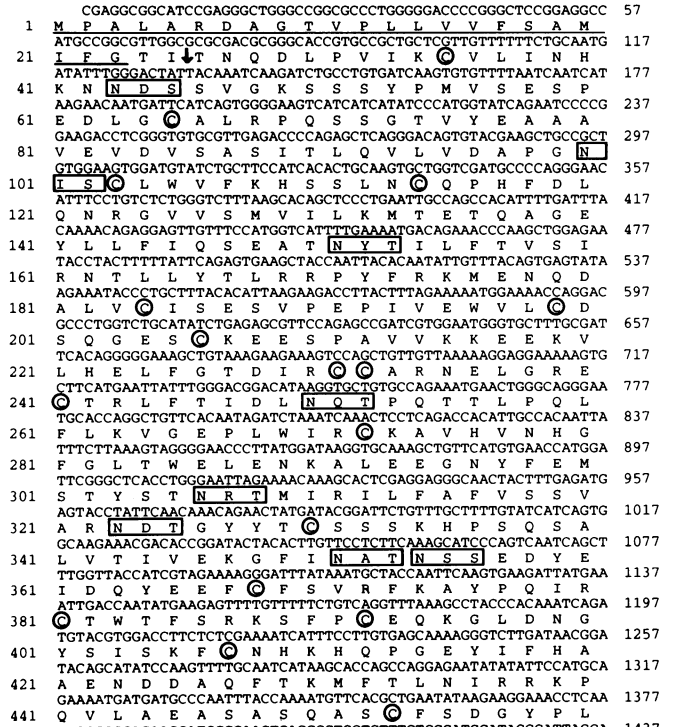
$CD34^+$ cells were suspended in 0.4 ml of Dulbecco's modified Eagle's medium supplemented with L-glutamine and 2% bovine calf serum. STK-1 S, Scr, or AS oligomers were added to the cell suspensions (time 0) and again 18 hr later. Thirty-six hours after initial exposure, the $CD34^+$ cells were cultured directly in colony-forming assays or on bone marrow stromal cell feeder layers (LTBMC; long-term bone marrow culture). For the LTBMC assay, cultures were agitated at weekly intervals and half of each culture was removed and assayed for granulocyte/macrophage colony-forming units (CFU-GM) (27–29).

RT-PCR was used for detection of STK-1 mRNA after exposure of $CD34^+$ cells to the oligonucleotides.

RESULTS

PCR amplification with degenerate kinase domain primers of human $CD34^+$ transcripts resulted in isolation of a fragment (HHSC-PTK.2A) with 82% nucleotide sequence homology and 97% amino acid homology to Flk-2/Flt-3. Flk-2/Flt-3 is a receptor TK cloned from murine fetal liver and testes by two groups (30, 31) that has been shown to be expressed in stem cell-enriched subsets of mouse fetal liver hematopoietic cells. Because of the possibility that Flk-2/Flt-3 serves as a hematopoietic stem cell growth factor receptor, we cloned the human homolog using the identified kinase fragment as a probe.

The resulting cDNA (Fig. 1) is 3.5 kbp and has an open reading frame of 2979 bp that includes a consensus Kozak translation initiation site next to the initiating ATG at nucleotide 58 (32). The predicted protein is 993 aa with a 25-aa signal peptide, a 516-aa extracellular domain that includes 22 cysteine residues and 10 potential asparagine-linked glycosylation sites, a 21-aa transmembrane domain, and a 431-aa cytoplasmic domain that includes the conserved TK domain



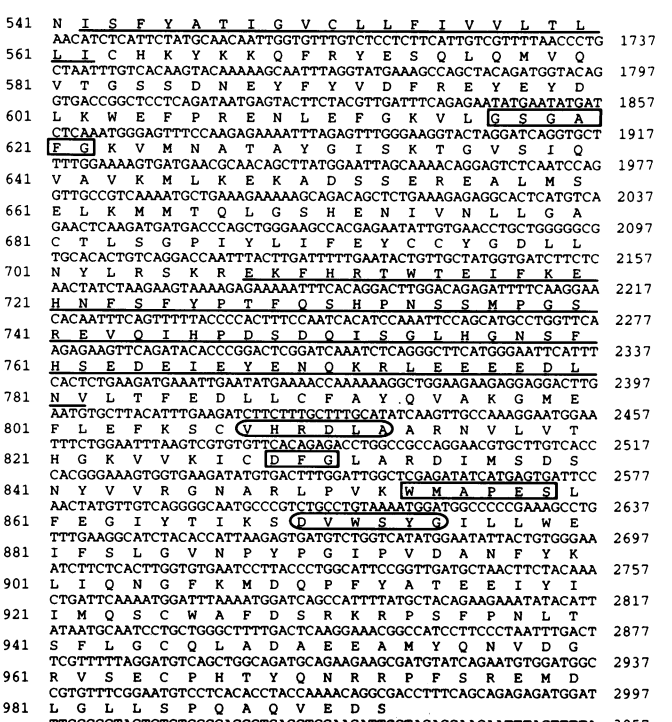


FIG. 1. Complete nucleotide and predicted amino acid sequence of STK-1 cDNA. Amino acids are numbered on the left of each column and nucleotides are numbered on the right. Underlined is the predicted signal peptide (aa 1–23) followed by the probable cleavage site marked with an arrow. The 22 cysteine residues of the extracellular domain are circled and the 10 potential asparagine-linked glycosylation sites are boxed. The single transmembrane spanning region is underlined (aa 542–562) as is the kinase insert region (aa 708–782) of the cytoplasmic domain. Several of the conserved domains of TKs including the GXGXXG (ATP binding domain), DFG, and WMAPES motifs are also boxed in the cytoplasmic domain. The peptides chosen for the design of the degenerate oligonucleotides used for the initial PCR are shown in ovals.

elements interrupted by a 75-aa kinase insert (10, 33, 34). Comparison of STK-1 with Flk-2/Flt-3 using the Genetics Computer Group program COMPARE shows conservation of all 22 cysteine residues and overall identity of 85% and similarity of 92% at the amino acid level (data not shown). Comparison with all other available TKs from the protein kinase catalytic domain data base shows that STK-1 is most closely related to type III growth factor receptor TKs, which include KIT, FMS, and platelet-derived growth factor receptor (11).

Expression of STK-1 was examined in subpopulations of human bone marrow. Preliminary studies showed that STK-1 was not a highly expressed message. Because of this and the small amounts of $CD34^+$ RNA obtained from human bone marrow, we used RT-PCR for these studies and confirmed the results with RNase protection. Total RNA was isolated from fractions of human bone marrow corresponding to $CD34^+$, $CD34^-$, and unfractionated mononuclear cells. Equal quantities of RNA and a no-RNA control were reverse transcribed with an oligo(dT) primer, and then PCR amplification was carried out with STK-1-specific primers. These primers were selected to span introns and give no signal from genomic DNA contamination (D.S., unpublished data). As shown in Fig. 2*A* (*Top*), bands corresponding to the expected target as well as a slightly larger band (possibly corresponding to partially or alternatively spliced message) were easily seen in the RNA from the $CD34^+$ cells, faintly seen in the RNA from total bone marrow, but not seen in the RNA from $CD34^-$ cells. As a control for the quality of the RNA preparations, the same reverse-transcribed RNA samples were amplified by using primers specific to KIT or FMS. As expected, all three RNA samples gave strong signals for KIT expression (Fig. 2*A Middle*), and expression of FMS was detected in whole marrow and the more mature $CD34^-$ fraction of bone marrow (Fig. 2*A Bottom*). These data demonstrate that unlike KIT and FMS, STK-1 expression is restricted to the $CD34^+$ LHSC-enriched population of human bone marrow.

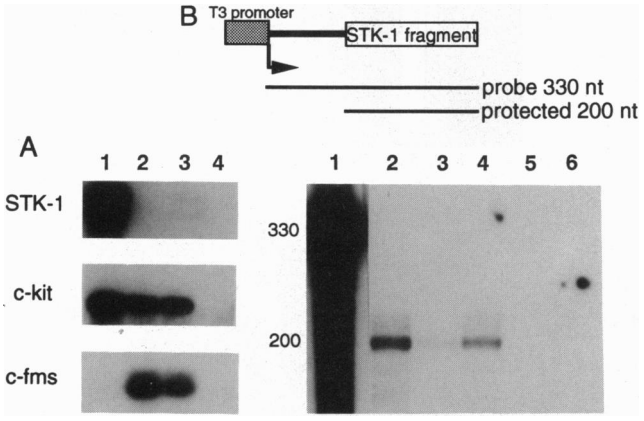


FIG. 2. (*A*) Comparison of STK-1 expression to KIT and FMS expression in bone marrow subpopulations by RT-PCR. Pairs of gene-specific primers (STK-1, KIT, or FMS) were used to amplify equal quantities of reverse-transcribed RNA samples from CD34-enriched (lane 1), CD34 double-depleted (lane 2), and total bone marrow (lane 3) fractions. Lane 4, no RNA. The reaction products were electrophoresed, transferred, and blotted with gene-specific probes. (*B*) Comparison of STK-1 expression in bone marrow subpopulations by RNase protection. (*Upper*) Schematic of STK-1 bacteriophage promoter construct with transcript size and predicted protected fragment. (*Lower*) RNase protection result. Lanes: 1, undigested probe; 2–6, digested products after hybridization with equal quantities of RNA from $CD34^+$ (lane 2), $CD34^-$ (lane 3), total bone marrow cells (lane 4), 20 μg of tRNA (lane 5), or no RNA (lane 6). Markers (nt) are from a sequencing ladder run in the same gel.

To confirm the results shown in Fig. 2*A* by a more quantitative method, we performed RNase protection experiments (Fig. 2*B*). Lane 1 shows the undigested probe of 330 bp. Lanes 5 and 6 show that 20 μg of tRNA or no RNA does not protect any of the probe from degradation. Lanes 2–4 show the result after addition of 5 μg of total RNA made from $CD34^+$, $CD34^-$, or unfractionated bone marrow mononuclear cells, respectively. A band of the expected size is seen with the greatest intensity in the $CD34^+$ lane, confirming that STK-1 expression is strongest in the LHSC-enriched fraction. It is likely that the signal in lane 3 comes from incomplete depletion of $CD34^+$ cells, since the $CD34^-$ population still contains 0.1–1% $CD34^+$ cells, varying from preparation to preparation. Aliquots of the same samples were electrophoresed, transferred to nitrocellulose, and hybridized with an actin gene probe confirming that approximately equal amounts of RNA were added (data not shown).

Mature hematopoietic cells fractionated from peripheral blood showed no expression by the RNase protection assay (data not shown), confirming that STK-1 is not expressed significantly in any major mature lymphohematopoietic cell type.

The predicted size of the STK-1 polypeptide is 113 kDa based on amino acid composition. Hyperimmune rabbit anti-STK-1 peptide antisera were then tested on Western blots of proteins extracted from human hematopoietic cell lines found to express STK-1 (D.S., unpublished data). Protein from a nonexpressing cell line (Molt-3) was used as a control. The anti-peptide antisera identified a doublet of bands, corresponding to 160 and 130 kDa, in the lanes containing protein from KMT2, ML-1, and KG1a cells (Fig. 3*A*). No bands were identified in the lanes containing proteins from Molt-3 cells. Preincubation of antibody with the immunizing peptide inhibited specific binding (Fig. 3*A*, + peptide lanes). The preimmunization bleed from this rabbit showed no bands at this position when used on a duplicate filter (data not shown).

3T3 cells transfected with the full-length STK-1 gene in the eukaryotic expression vector pcDNAI/neo (Invitrogen) also expressed the immunoreactive bands (Fig. 3*B*).

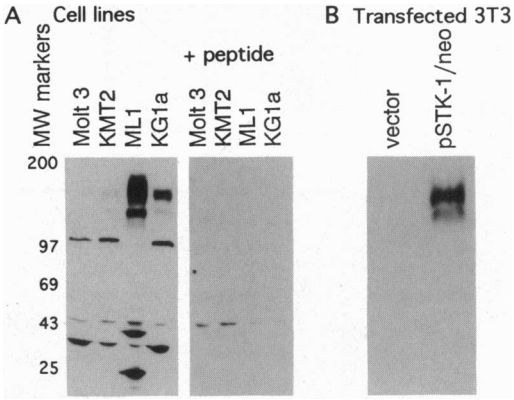


FIG. 3. Western blot of proteins from STK-1-expressing and nonexpressing cell lines. (*A*) Approximately equal amounts of protein extracted from the indicated cell lines were electrophoresed through 4–12% polyacrylamide gels and transferred to nitrocellulose. After blocking, the blot was incubated with affinity-purified rabbit polyclonal antibody (5 μg/ml) raised against a peptide from the kinase insert region. Lanes marked + peptide were treated the same as the lanes on the left but the peptide used to generate antibody was added to the antibody at 20 μg/ml before incubating antibody with the nitrocellulose filter. After washing, filters were exposed to anti-rabbit immunoglobulin conjugated to peroxidase and developed (ECL; Amersham). Numbers on left are kDa. (*B*) Equal amounts of protein from 3T3 cells transfected with either vector alone or vector expressing STK-1 were treated as described above.

To determine the biologic function of STK-1 in normal hematopoietic progenitor cells, disruption experiments were carried out with STK-1 AS ODNs using *in vitro* colony-forming assays. $CD34^+$ cells were exposed to increasing doses of AS ODNs and to doses of S and Scr ODNs equivalent to the highest AS dose used. Neither S nor Scr sequence ODN inhibited colony formation. In contrast, dose-dependent inhibition was noted with the AS ODN. At the highest doses used, CFU-GM colony formation was inhibited 53% ($P < 0.001$), and 46% ($P = 0.003$), respectively, when the US and DS mRNA sequences were targeted (Fig. 4*A*). The TS sequence gave no significant inhibition. When treated in an identical manner, STK-1 US inhibited erythroid burst-forming units (BFU-E) 57% ($P < 0.001$), while STK-1 DS inhibited 40% ($P = 0.002$) (data not shown). Again, targeting the translational start site was without effect. Similar inhibition was also seen when the experiments were carried out on adherent and T-lymphocyte-depleted mononuclear cells.

In an effort to determine the role of STK-1 in a less-mature cell population, we examined the effect of STK-1 ODNs on CFU-granulocyte/erythrocyte/monocyte/megakaryocyte (GEMM) (Fig. 4*B*). Colony formation by this progenitor was decreased by ≈80% ($P = 0.001$) targeting the US sequence and 62% ($P = 0.07$) targeting the DS sequence. Cells cultured in the presence of STK-1 TS were not inhibited in a statistically significant manner ($P = 0.18$). As noted before, neither S nor Scr sequences inhibited CFU-GEMM colony formation.

In aggregate, these results suggested that STK-1 function was necessary for growth of myeloid and erythroid progenitor cells and that early progenitor cells (CFU-GEMM) might be more dependent on the putative signaling function of this kinase than later progenitor cells (CFU-GM and BFU-E). These results also suggested that the US sequence of the message was likely the best target for AS-mediated disruption of STK function.

To test the hypothesis that STK-1 function was most important for early progenitor cell development, we established LTBMCs and then determined the ability of stem-like progenitors in such cultures to give rise to CFU-GM after exposure to STK-1 US ODNs. As shown in Fig. 4*C*, the ability of LTBMCs to give rise to CFU-GM was inhibited almost immediately and was decreased ≈95% by week 3 and 100% by week 4. Exposure of $CD34^+$ cells to STK-1 AS ODN under these conditions resulted in the absence of STK-1 message, as assayed by RT-PCR (Fig. 4*D*). This confirms that the AS effects were due to the inhibition of STK-1 expression. These results suggest that the STK-1 receptor is likely most important in very early hematopoietic cells close to or at the level of the hematopoietic stem cell.

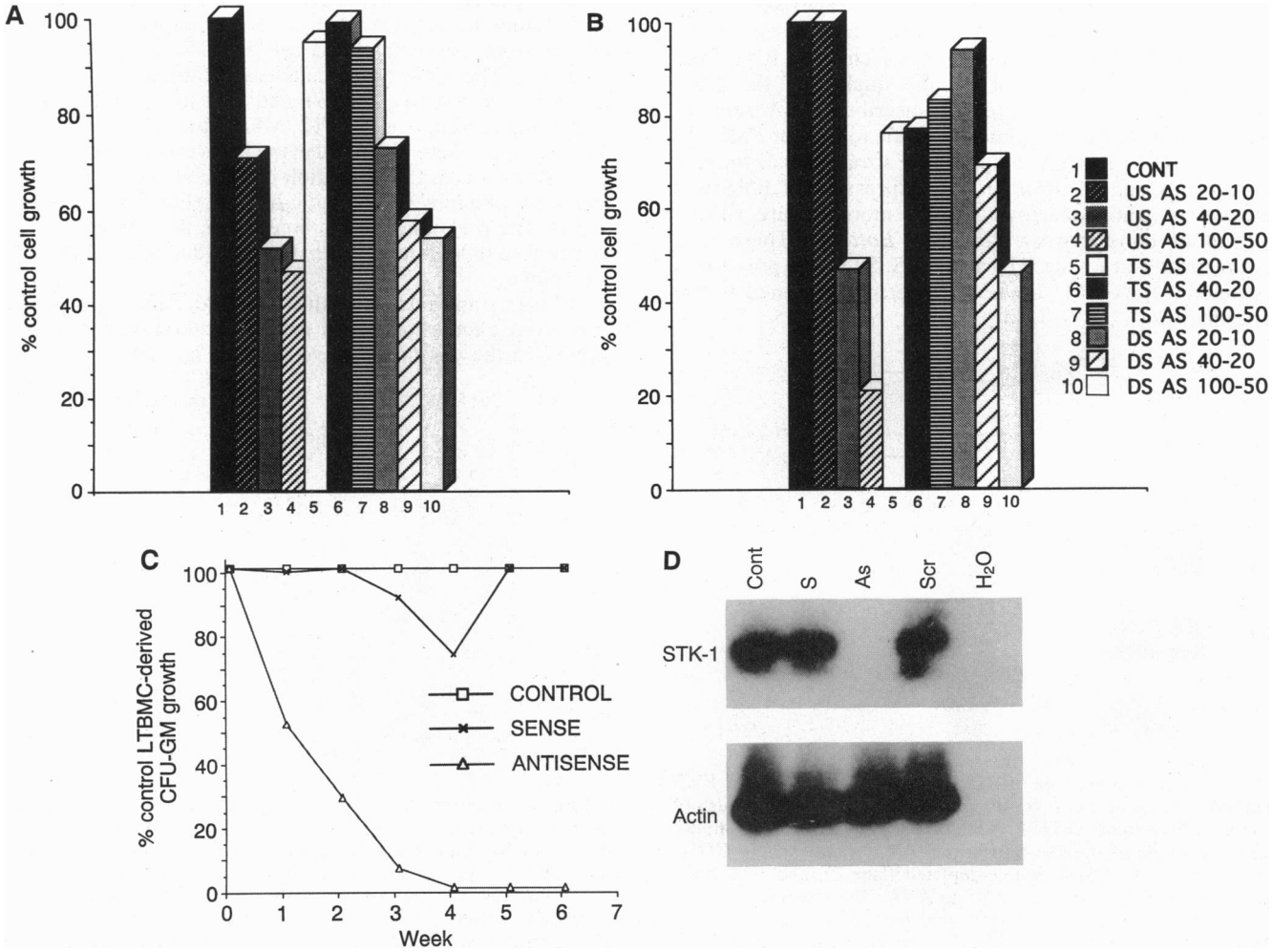


FIG. 4. Effect of STK-1 AS ODNs on normal hematopoietic cell colony growth. $CD34^+$ cells were exposed to STK-1 ODNs at the indicated concentrations (e.g., US AS 20–10 indicates US AS oligomer at 20 μg/ml for 18 hr and then at 10 μg/ml for an additional 18 hr) and then assayed for CFU-GM (*A*) or CFU-GEMM (*B*) colonies. (*C*) $CD34^+$ cells were exposed to the US AS, S, and control (Scr) oligomers at 100 μg/ml for 18 hr and then at 50 μg/ml for an additional 18 hr. Treated cells were plated on irradiated bone marrow stromal cell feeder layers and assayed for CFU-GM colonies at weekly intervals. (*D*) Result of RT-PCR to assess STK-1 expression after exposure of $CD34^+$ cells to the ODN as in *C*.

DISCUSSION

We have cloned the cDNA for STK-1, the human homolog for murine Flk-2/Flt-3. The sequence of murine Flk-2 and Flt-3 differed in several locations (30, 31). By comparison to the reported murine cDNAs, the human sequence is homologous to the C-terminal region reported for Flt-3 as well as the other amino acid differences except at aa 242 where, like Flk-2, it has a cysteine residue. Recently, a third group has reported cloning the murine cDNA and their sequence agrees with the STK-1 C terminus and single amino acid differences (35).

Flk-2 has been shown to be expressed on a population of cells enriched in hematopoietic stem cell activity (30). In this paper, we have shown by both RT-PCR and the more quantitative RNase protection assay that STK-1 expression seems limited to the $CD34^+$ cell fraction of human bone marrow. It will be interesting to determine whether STK-1 is expressed in a cell population reported to contain highly enriched pluripotent hematopoietic stem cells ($CD34^+/lin^-/CD38^-$) (36) and in the even more primitive mesenchymal stem cell-enriched cell fraction ($CD34^+/lin^-/CD38^-/HLADR^-$) recently suggested to give rise to both the hematopoietic and stromal elements of marrow, as well as to other lineages (37).

RT-PCR of $CD34^+$ RNA results in two bands (see Fig. 3*A*). Thus, STK-1 may have alternatively expressed messages, as has been seen for a number of other receptors, often resulting in expression of soluble receptor. It is of note that other STK-1 PCR primer pairs do not give rise to a doublet. An insertion in this region could truncate the receptor prior to the transmembrane domain, resulting in soluble receptor.

Most cell-surface receptors are heavily glycosylated and STK-1 appears to be heavily glycosylated as well. Based on its amino acid composition, its expected molecular mass would be 113 kDa, but it migrates as a doublet of 130 and 160 kDa. This is probably due to the 10 potential asparagine-linked glycosylation sites in the STK-1 extracellular domain. The murine homolog is expressed as a similar doublet, and the higher molecular weight form was shown to depend on asparagine-linked glycosylation (35, 38). The level of STK-1 expression and degree of glycosylation of the receptor varies significantly between cell lines. Whether or not these differences result in functional differences is not yet known.

Birg *et al.* (39) have recently demonstrated expression of Flt-3 in most leukemias. We have seen similar results (D.S., unpublished data). It will be of interest to determine whether overexpression, a mutated receptor, or autocrine loops involving STK-1 play a role in any leukemias.

STK-1/Flk-2/Flt-3 is a member of the type III receptor TK family, which also includes KIT, FMS, and platelet-derived growth factor receptor. These receptors have been shown to be important in the growth and/or differentiation of a variety of cells in which they are expressed. A chimeric receptor composed of the extracellular domain of FMS fused to the transmembrane and cytoplasmic domains of Flt-3 was recently shown to result in colony-stimulating factor 1-dependent growth of cells that had been transfected with this construct (38). Because STK-1 expression in hematopoiesis seems limited to the $CD34^+$ stem/progenitor cell fraction of both murine and human hematopoietic cells, STK-1 is likely to be involved in growth and/or differentiation of these cells. The STK-1 AS experiments presented here demonstrate a significant inhibition of colony-forming activity, most marked in the LTBMC assay. This indicates that STK-1 may indeed be involved in regulation of proliferation of pluripotent LHSCs. It is unclear (yet) whether the mechanism of inhibition by AS treatment is the death or the commitment to differentiation of the progenitor/stem cells.

Full evaluation of the expression and role of STK-1 in human hematopoiesis awaits the discovery of its ligand and production of anti-STK-1 monoclonal antibodies. If STK-1 antibody enables the purification of stem cells and its ligand allows for the expansion of these cells, they will find practical use in clinical medicine.

The authors would like to thank R.S., L.C.S., and M.S. for a careful reading of the manuscript. This work was supported by grants from the Mildred Mindell Cancer Foundation (D.S.), Robert F. Goldhammer Foundation (D.S.), Sandoz Clinician Scientist Award (D.S.), and ImClone Systems, Inc. (D.S., C.I.C.); by American Chemical Society Grant CH-480A (C.I.C.); and by National Institutes of Health Grants CA36896 (A.M.G.), CA54384 (A.M.G.), and CA51083 (A.M.G.).

1. Till, J. E. & McCulloch, E. A. (1980) *Biochem. Biophys. Res. Commun.* **605**, 431–459.
2. Spangrude, G. J., Heimfeld, S. & Weissmann, L. L. (1988) *Science* **241**, 58–62.
3. Civin, C. I., Strauss, L. C., Brovall, C., Fackler, M. J., Schwartz, J. F. & Shaper, J. H. (1984) *J. Immunol.* **133**, 157–165.
4. Strauss, L. C., Rowley, S. D., La Russa, V. F., Sharkis, S. J., Stuart, R. K. & Civin, C. I. (1986) *Exp. Hematol. (NY)* **14**, 878–886.
5. Civin, C. I. & Loken, M. R. (1987) *Int. J. Cell Cloning* **5**, 1–13.
6. Berenson, R. J., Andrews, R. G., Bensinger, W. I., Kalamasz, D., Knitter, G., Buckner, C. D. & Bernstein, I. D. (1988) *J. Clin. Invest.* **81**, 951–960.
7. Berenson, R. J., Bensinger, W. I., Hill, R. S., Andrews, R. G., Garcia-Lopez, J., Kalamaz, D. F., Still, B. J., Spitzer, G., Buckner, D. & Bernstein, I. D. (1991) *Blood* **77**, 1717.
8. Williams, M. E. & Quesenberry, P. J. (1992) *Hematol. Pathol.* **6**, 105–124.
9. Pawson, T. & Bernstein, A. (1990) *Trends Genet.* **6**, 350–356.
10. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
11. Hanks, S. K. & Quinn, A. M. (1991) *Methods Enzymol.* **200**, 38–62.
12. Hunter, T. (1991) *Methods Enzymol.* **200**, 3–37.
13. Sherr, C. J. (1990) *Blood* **75**, 1–12.
14. Chabot, B., Stevenston, D. A., Chapman, V. M., Besmer, P. & Bernstein, A. (1988) *Nature (London)* **335**, 88–89.
15. Geissler, E. N., Ryan, M. A. & Housman, D. E. (1988) *Cell* **55**, 185–192.
16. Wilks, A. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1603–1607.
17. Strauss, L. C., Trischmann, T. M., Rowley, S. D., Wiley, J. M. & Civin, C. I. (1991) *Am. J. Pediatr. Hematol. Oncol.* **13**, 217–221.
18. Civin, C. I., Strauss, L. C., Fackler, M. J., Trischmann, T. M., Wiley, J. M. & Loken, M. R. (1990) *Bone Marrow Purging and Processing*, eds. Gross, S., Gee, A. & Worthington-White, D. (Liss, New York), pp. 387–402.
19. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
20. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
21. Frohman, M. A., Dush, M. K. & Martin, G. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8998–9002.
22. Jain, R., Gomer, R. H. & Murtagh, J. J., Jr. (1992) *BioTechniques* **12**, 58–59.
23. Southern, E. M. (1975) *J. Mol. Biol.* **98**, 503–517.
24. Feinberg, A. P. & Vogelstein, B. (1984) *Anal. Biochem.* **132**, 6–13.
25. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
26. Burnette, E. H. (1981) *Anal. Biochem.* **112**, 195–203.
27. Ratajczak, M., Luger, S., DeReil, K., Abrahm, J., Calabretta, B. & Gewirtz, A. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1710–1714.
28. Ratajczak, M., Hijiya, N., Cantani, L., DeReil, K., Luger, S. L., McGlave, P. & Gewirtz, A. (1992) *Blood* **79**, 1956–1961.
29. Gewirtz, A. & Calabretta, B. (1988) *Science* **242**, 1303–1306.
30. Matthews, W., Jordan, C. T., Wiegand, G. W., Pardoll, D. & Lemischka, I. R. (1991) *Cell* **65**, 1143–1152.
31. Rosnet, O., Marchetto, S., DeLapeyriere, O. & Birnbaum, D. (1991) *Oncogene* **6**, 1641–1650.
32. Kozak, M. (1986) *Cell* **44**, 283–292.
33. Watson, M. E. E. (1984) *Nucleic Acids Res.* **12**, 5145–5164.
34. Von Heijne, G. (1983) *Eur. J. Biochem.* **133**, 17–21.
35. Lyman, S., James, L., Zappone, J., Sleath, P., Beckmann, M. & Bird, T. (1993) *Oncogene* **8**, 815–822.
36. Terstappen, L. W. M. M., Huang, S., Safford, M., Lansdorp, P. & Loken, M. R. (1991) *Blood* **77**, 1218–1227.
37. Huang, S. & Terstappen, L. W. M. M. (1992) *Nature (London)* **360**, 709–710.
38. Maroc, N., Rottapel, R., Rosnet, O., Marchetto, S., Lavezzi, C., Mannoni, B., Birnbaum, D. & Dubreuil, P. (1993) *Oncogene* **8**, 909–918.
39. Birg, F., Courcoul, M., Rosnet, O., Bardin, F., Pebusque, M., Marchetto, S., Tabilio, A., Mannoni, P. & Birnbaum, D. (1992) *Blood* **80**, 2584–2593.