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# Presence and Expression of a Novel Variant Form of ST2 Gene Product in Human Leukemic Cell Line UT-7/GM[1]

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**A novel variant cDNA from the human ST2 gene other than ST2 or ST2L was identified and tentatively named ST2V. Alternative splicing inserts a new exon which leads to a change in the C-terminal portion of ST2, causing it to gain a hydrophobic tail instead of losing the third immunoglobulin-like domain. ST2V is expressed in human leukemic cell line UT-7 and its sublines UT-7/GM, UT-7/EPO, and UT-7/TPO, in addition to human helper T cell line 5C10. The amount of ST2V mRNA is greatly diminished when UT-7/GM cells are induced to differentiate into either erythroblastic or megakaryoblastic phenotypes. The possible roles of the ST2V in growth and differentiation are intriguing.** 

ST2 was originally identified as a primary response gene that was highly induced by the addition of serum to the quiescent BALB/c-3T3 murine fibroblast (1). It was also designated as T1, DER4, Fit-1, and had been investigated to clarify its biological activities (2–4).

Two distinct types of the ST2 gene products, a soluble secreted form (ST2) and a transmembrane form (ST2L), had been cloned (1, 5). These were reported to be generated by alternative splicing in a rat system (4). ST2L was very similar to IL-1RI in structure, but it did not bind IL-1$\alpha$, $\beta$, receptor antagonist; therefore it was suggested that ST2L is a member of the IL-1R family, responding to a yet unknown ligand (5, 6). In this context, the elucidation of roles of the soluble secreted form, ST2, for modification of signal transduction of ST2L receptor is intriguing.

A strikingly wide variety of human cells have been shown to express ST2 gene products, including hematopoietic cells in various stages of differentiation, a population of the peripheral blood mononuclear cells from healthy individuals, glioblastoma and astrocytoma cell lines, and colon cancer cells in addition to fibroblastic cell lines (6). This evidence suggests that ST2 gene products have distinct physiological roles in different cell systems.

Recently, we reported the constitutive expression of ST2L in a murine Th2 cell line, and the all-or-none type induction of ST2 when the cells were stimulated with PMA and calcium ionophore A23187 that mimic the antigenic stimuli (6). The expression was limited in Th2 cells, and has not so far been found in Th1 cells. Furthermore, two reports recently suggested the possible role of ST2L in exacerbation of bronchial asthma in a murine model system (7, 8). Taken together, these studies indicate that the functions of ST2 gene products are not only in growth control but also in the immunological response via helper T cells.

In the course of cloning human ST2 cDNA using the cDNA library from the 5C10 helper T cell line, we found a third type of ST2 gene product that was neither ST2 or ST2L. Here we report the presence of a novel variant form of human ST2 named tentatively ST2V. The expression of ST2V is greatly diminished when human leukemic cell line UT-7/GM is induced to differentiate into both erythroblastic and megakaryoblastic phenotypes.

Abbreviations used: dCTP, deoxycytidine 5′-triphosphate; dNTPs, deoxyribonucleotide 5′-triphosphates; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; EPO, erythropoietin; FCS, fetal calf serum; GAPDH, glceraldehyde 3-phosphate dehydrogenase; GM-CSF, granulocyte macrophage colony stimulating factor; IL-3, interleukin 3; IMDM, Iscove's modified Dulbecco's medium; PMA, phorbol myristate acetate; RT-PCR, reverse transcription polymerase chain reaction; SDS, sodium dodecyl sulfate; TPO, thrombopoietin.

[1] The nucleotide sequence data presented here will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the Accession No. AB029084.

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## MATERIALS AND METHODS

*Materials.* The cDNA library from human helper T cell line 5C10 was a kind gift from Dr. T. Yokota (The Institute of Medical Science, The University of Tokyo) (9). Recombinant human EPO was a gift from the Life Science Research Institute of the Snow Brand Milk Company (Tochigi, Japan). Recombinant human TPO was provided by the Kirin Brewery Co., Ltd. (Gunma, Japan). Recombinant human GM-CSF was supplied by the Sumitomo Pharmaceutical Company (Osaka, Japan).

The primers used for RT-PCR analysis were the following: a, 5′-CAGGTCCTTCACGGTCAAGG-3′ corresponding to the 3′ edge of exon 5 in the forward direction (591 → 610 in Ref. 10) (see text and Fig. 2A); b, 5′-TCAAGGCCATTGATGATGTT-3′ corresponding to the 3′ edge of exon 5E in the reverse direction (Fig. 2A); c, 5′-TTTGGTGTCAGAGTTTCTGC-3′ corresponding to the 5′ edge of exon 5E in the forward direction (Fig. 2A); d, 5′-CAGAGA-AAAGCCTTGCTCAT-3′ corresponding to the 5′ edge of exon 6 in the reverse direction (630 → 611) (Fig. 2A); e, 5′-GAAAAAACGC-AAACCTAACT-3′ corresponding to the initial part of exon 7 in the forward direction (683 → 702 in Ref. 10); f, 5′-TCAGAAACA-CTCCTTACTTG-3′ corresponding to the ST2 translation termination part of exon 8 in the reverse direction (987 → 968 in Ref. 10); g, 5′-GTGACGGCGACCAGGTCCTT-3′ in the forward direction corresponding to the upstream region of the inserted sequence (580 → 599 in Ref. 10). GAPDH forward: 5′-TGGGGAAGGTGAAGGTCGG-AGTCAACGG-3′ (2 → 29 in Ref. 11). GAPDH reverse: 5′-GGC-AGGTCAGGTCCACCACTGACACGTTG-3′ (742 → 714 in Ref. 11).

*Cell culture.* The original UT-7 cells were maintained in liquid culture with IMDM (GIBCO Laboratories, Grand Island, NY) supplemented with 10% FCS (GIBCO) and 1 ng GM-CSF/ml by replacement of three-fourths of the medium every 3 or 4 days (12). The UT-7/GM cells were maintained in IMDM plus 10% FCS with 1 ng of GM-CSF/ml (13). The UT-7/EPO and UT-7/TPO cell lines were continuously maintained in IMDM containing 10% FCS with 1 U of EPO/ml and 10 ng of TPO/ml, respectively (14, 15).

*RT-PCR analysis.* Total cellular RNA was extracted from UT-7 cell lines using ISOGEN (Nippon Gene) according to the manufacturer's instructions. To synthesize the first-strand cDNA, 5 $\mu$g of total RNAs were reverse-transcribed with 4 $\mu$M of random hexamer, 100 $\mu$M each of four dNTPs, 200 units of RNase inhibitor (Toyobo) and 200 units of SuperScript II RNase H$^-$Reverse Transcriptase (BRL) in a buffer containing 50 mM Tris-HCl (pH 8.3 at 20°C), 75 mM KCl, 3 mM $MgCl_2$, and 10 mM DTT in a total volume of 25 $\mu$l at 41°C for 60 min. The synthesized first-strand cDNA was precipitated by ethanol and dissolved in 10 $\mu$l of distilled water. The PCR was performed using a 0.5-$\mu$l solution of cDNA as a template in a total volume of 20 $\mu$l that contained 0.4 $\mu$M each of two primers, 0.4 $\mu$M each of GAPDH forward and reverse primers, 200 $\mu$M each of the four dNTPs, and 0.4 $\mu$l of 50xAdvantage cDNA Polymerase Mix (Clontech) in the buffer recommended by the manufacturer. Thirty cycles of the reaction at 94°C for 1 min, 60°C for 2 min, and 72°C for 3 min were carried out in the DNA Thermal Cycler 480 (Perkin Elmer Cetus). Five $\mu$l of the sample was developed by electrophoresis using 5% polyacrylamide gel or 1% agarose gel and then visualized by ethidium bromide staining.

*Quantitative RT-PCR with Southern blotting analysis.* RT-PCR was carried out as described above except for 20 cycles of the reaction instead of 30 cycles. Five $\mu$l of each of the PCR products was subjected to electrophoresis on a 1% agarose gel and transferred to a nylon membrane (Hybond N+; Amersham) by Southern blotting (16). For the analysis of ST2V expression, the 170-bp DNA fragment, corresponding to exon 5E (see text), was made by PCR using primers b and c, and purified through UltraClean (MO BIO Laboratories, Inc., USA) according to the manufacturer's protocol. For the analysis of ST2 expression, a 617-bp fragment that was cut out from the human ST2 cDNA with *Pvu* II and *Xba* I was purified (10). The fragment was radiolabeled with [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol; Amersham) by means of a Ready-to-Go random primers labeling kit (Pharmacia) and used as a probe. DNA on the filter was hybridized overnight at 65°C in a mixture of 0.5 M Church phosphate buffer (17), 1 mM EDTA, 7% SDS, and $^{32}$P-labeled denatured probe DNA. The filter was washed three times in 40 mM Church phosphate buffer containing 1% SDS at 65°C for 5 min, and then it was finally washed in the same solution for 15 min. Autoradiography was carried out by exposing the membrane to an X-ray film at −80°C. We used a BAS2000 analyzer (Fuji Film, Japan) for quantitative analysis. GAPDH was used as an internal control.

## RESULTS AND DISCUSSION

### *The Structure of the Variant Form of the ST2 Gene Product*

In the course of cloning human ST2 cDNA from the cDNA library of a human helper T cell line, 5C10, we found another distinct cDNA clone (10). The nucleotide sequence of the cDNA was determined as described previously (1).

The only difference in the nucleotide sequences between the new clone ST2V and the original human ST2 clone was the presence of 170 bp in the ST2V cDNA that was inserted in the middle of the ST2 cDNA (Fig. 1A). The insertion of the sequence resulted in the introduction of a new hydrophobic tail and premature termination because of the presence of a TGA termination codon at the end of the insertion (Figs. 1A and 1B). The schematic representation shows the absence of the third immunoglobulin-like domain in ST2V (Fig. 1C).

Recently we reported the presence of two distinct promoter regions in the human ST2 gene. In the case of UT-7/GM cells, the major promoter used to express both ST2 and ST2L mRNAs is the upstream one followed by non coding exon 1a (18). On the other hand, the cloned ST2V cDNA contained proximal exon 1b (Fig. 1A) (18, 19). Further study is necessary to assess whether ST2V mRNA is transcribed mainly from the proximal promoter or not.

### *Partial Exon-Intron Organization of ST2V*

To rule out the possibility that the inserted nucleotides were simply derived from an unspliced intron, PCR was carried out using human ST2 genomic DNA as a template (10) and four primers, a, b, c, and d, whose nucleotide sequences were shown in Materials and Methods (see also the arrows in Fig. 2A). The PCR product using primers b and c was 170 bp in length, suggesting that there was no intron between primers b and c. Together with the data in Fig. 2B, this clarified that a new exon, exon 5E (extra), was the origin of the inserted nucleotides in the case of ST2V cDNA (Fig. 2A). Thus, the ST2 and ST2V were generated by an alternative splicing mechanism.

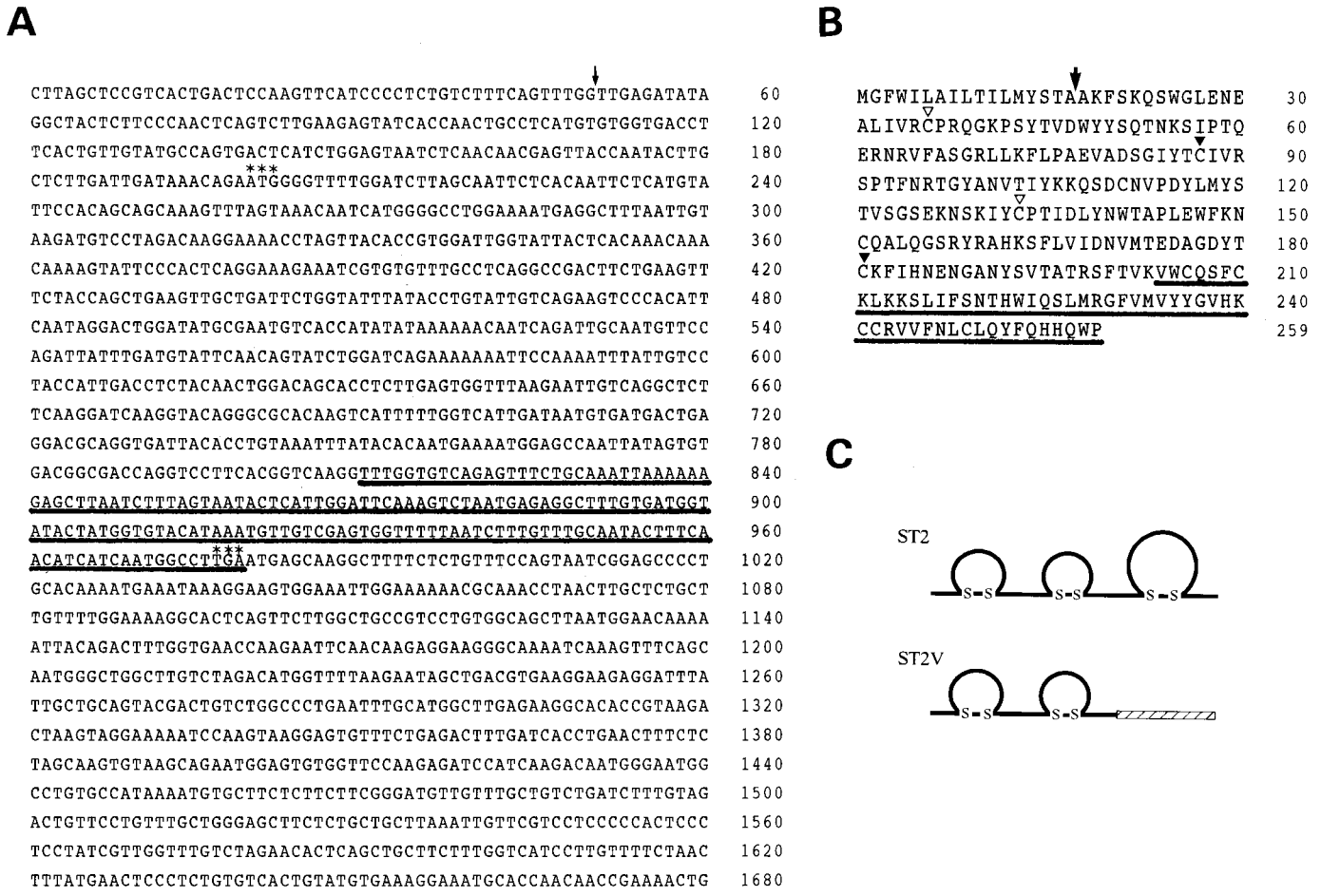


**FIG. 1.** The structure of the novel variant form of human ST2 gene product. (A) The nucleotide sequence of ST2V cDNA cloned from the cDNA library of 5C10 (10). The novel inserted region is underlined. The initiation and termination codons are indicated by asterisks. The arrow indicates the boundary between exon 1b and 2. (B) The amino acid sequence deduced from the nucleotide sequence shown in Panel A. The underlined region corresponds to the amino acids unique to ST2V. The N-terminus and C-terminus of each immunoglobulin-like domain are indicated by the open and closed triangles, respectively. The arrow indicates the possible cleavage site of the signal peptide according to von Heijne's rule (21). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr. (C) Schematic representation of the structure of ST2 and ST2V protein. The shaded area indicates the distinct tail of the ST2V.

*Expression of ST2V in Various Sublines of Human Leukemic Cell Line UT-7*

We chose RT-PCR for studying the expression of ST2V in various cell lines. Since PCR can amplify a tiny amount of contaminated chromosomal DNA in the purified total RNA preparation, it is very important to design primers to pick up specific signals derived from ST2V mRNA. We chose primer sets c and d (Fig. 2A), which would generate a 190-bp product in the case of ST2V mRNA, an approximately 1-kb product in the case of contaminated chromosomal DNA, and no product in the case of ST2 mRNA (see lanes 5 to 7, Fig. 3A). Using this RT-PCR system, we found the presence of ST2V mRNA in various sublines of the UT-7 human leukemic cell line in addition to the 5C10 helper T cell line.

Nucleotide sequence analysis on the RT-PCR products, which were amplified using the primer set of oBC009 and oBC010 (18), revealed that identical ST2V mRNA was expressed in UT-7 cells. UT-7 is the original human leukemic cell line and absolutely dependent on IL-3 or GM-CSF to grow (12). It has been reported to produce both ST2 and ST2L proteins (20). UT-7/EPO is derived from UT-7, and its growth is supported by erythropoietin but not by GM-CSF or IL-3 (14). UT-7/GM is also derived from UT-7 and dependent on the presence of GM-CSF to proliferate (13). UT-7/TPO was established from UT-7/GM by culture at a low concentration of TPO, and it can be maintained by TPO alone (15).

As shown in Fig. 3A, the amount of ST2V mRNA was different among the 4 cell lines. The original UT-7

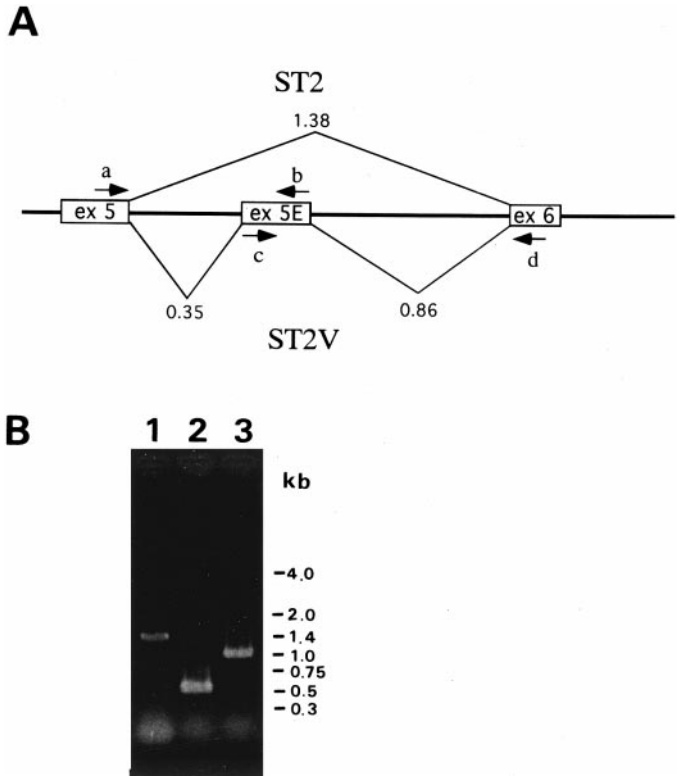


**FIG. 2.** Partial exon-intron organization of ST2 and ST2V. (A) Schematic representation of exon-intron organization based on the data of (B). Arrows indicate the positions and directions of the 4 primers used. Exons are boxed. A new exon containing 170 bp was designated as exon 5E (extra). The distance between exons was calculated by plotting the mobility of a PCR product on the calibration line in semi-log scale, and it is represented in kb. (B) The PCR products using a and d primers (lane 1), a and b primers (lane 2), and c and d primers (lane 3) were developed by 1% agarose gel electrophoresis and visualized by ethidium bromide staining. The positions of the DNA markers are shown on the right side.

contained a small amount of ST2V mRNA compared to the downstream subline UT-7/GM. To accomplish quantitative measurement, after reverse transcription, we performed 20 cycles of PCR and subsequent Southern blotting analysis as described in Materials and Methods. Twenty cycles were chosen because the amount of PCR product of GAPDH in the same reaction condition was in a linear range at 20 cycles (data not shown). As shown in Fig. 3C, the signal was the strongest in the most downstream subline UT-7/TPO (15). The different amount of expression of ST2V mRNA among the 4 cell lines is reproducible in 4 independent experiments, but the implication is unclear at present.

To make a comparison, we tried to measure the amount of ST2 mRNA among the 4 cell lines. Since there is no unique sequence derived from ST2 only, we chose the forward primer e, corresponding to the initial part of exon 7, and the reverse primer f, corresponding to the ST2 translation termination part of exon 8, which were used in the former study (10) (Fig. 3B). The product of this PCR reaction consisting of 305 bp is common to ST2 and ST2V (see lanes 5 and 6, Fig. 3B). However, the major constituent of this band was derived from ST2, as shown by the fact that the mRNA ratio of ST2V/ST2 in UT-7/GM cells was 0.11 and 0.16 in two independent experiments. This ratio was measured by the Southern blotting procedure as described above using the forward primer g, which corresponds to the upstream region of the inserted sequence, and the reverse primer f, which enabled us to detect the PCR products from these two mRNAs in different mo-

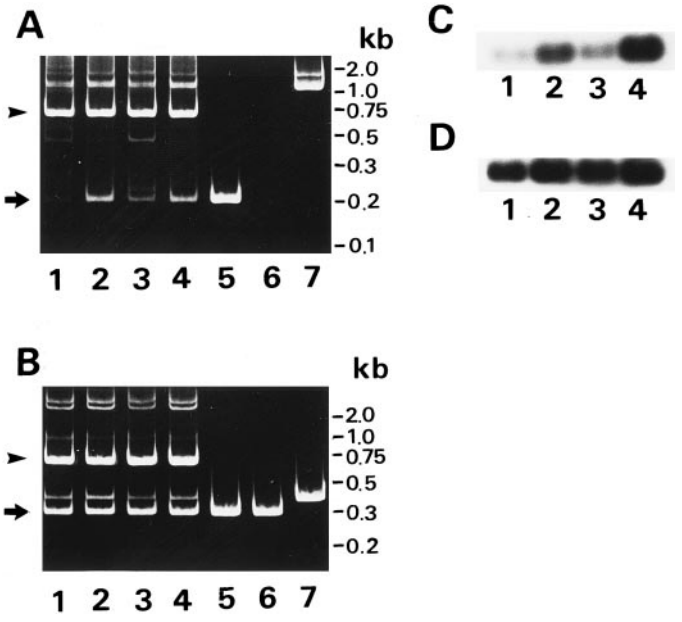


**FIG. 3.** Expression of ST2V and ST2 mRNAs in various UT-7 sublines. (A) Expression of ST2V mRNA. Total RNA was extracted from UT-7 (lane 1), UT-7/GM (lane 2), UT-7/EPO (lane 3), or UT-7/TPO (lane 4). Reverse transcription and PCR of 30 cycles, using primers c and d (see Fig. 2A) together with GAPDH forward and reverse primers, were carried out as described in Materials and Methods. For the control experiment, PCR was carried out in 20 $\mu$l of reaction mixture containing 0.1 $\mu$g of pUC19 plasmid carrying ST2V cDNA (lane 5), 0.1 $\mu$g of pEF-BOS-ST2H plasmid carrying ST2 cDNA (20) (lane 6), or 0.1 $\mu$g of $\lambda$DASH phage DNA carrying human ST2 genomic ST2 fragment (10) (lane 7) as a template. PCR products were developed by 5% polyacrylamide gel electrophoresis and stained with ethidium bromide. The arrow indicates the position of the PCR product specific for ST2V mRNA. The arrowhead indicates the position of the PCR product from GAPDH mRNA for internal control. (B) Expression of ST2 and ST2V mRNAs. The experiment was carried out in the same condition as in Panel A except for using primers e and f instead of c and d (see Materials and Methods and Ref. 10) for PCR. (C) Quantitative RT-PCR with Southern blotting analysis was performed as described in Materials and Methods, using primers c and d and exon 5E fragment as a probe. The autoradiogram of 190-bp products from UT-7 (lane 1), UT-7/GM (lane 2), UT-7/EPO (lane 3), and UT-7/TPO (lane 4) is shown. (D) Primers e and f and a probe of a 617-bp fragment of ST2 cDNA (see Materials and Methods) were used in the same experiment as in (C) to detect the expression of ST2 and ST2V mRNAs. The autoradiogram of a 305-bp product is shown for each cell line as in (C).

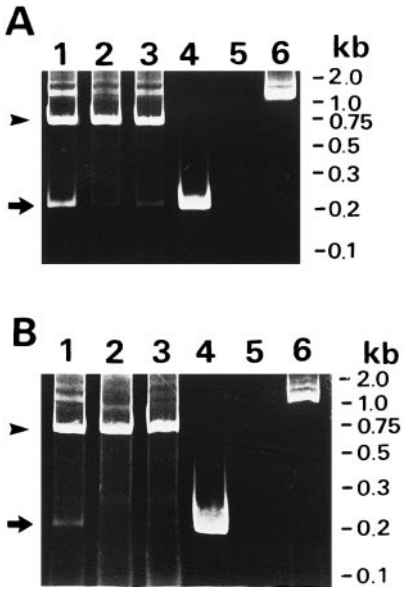


**FIG. 4.** The expression of ST2V mRNA after differentiation of UT-7/GM cells. (A) UT-7/GM cells were cultured in the presence of 10 ng/ml of GM-CSF (lane 1), 10 U/ml of EPO (lane 2), or 100 ng/ml of TPO (lane 3), for 5 days. RT-PCR was performed using primers c and d as in Fig. 3A. Control lanes for ST2V (lane 4), ST2 (lane 5), and genomic ST2 (lane 6) were included as in Fig. 3A. The arrow indicates the position of a 190-bp product from ST2V. The arrowhead indicates the position of a 741-bp product from GAPDH as an internal control. (B) The culture was continued for 2 weeks, and the cells were processed for RT-PCR analysis as in (A).

bilities (data not shown). Therefore, the expression of ST2 mRNA could be judged to be nearly the same among the 4 cell lines (Fig. 3D).

The different expression level of ST2V mRNA among the various cell lines in contrast to the relatively constant expression level of ST2 mRNA may suggest some regulatory role of ST2V in hematopoiesis.

### *Decrease of the Amount of ST2V Expression during the Induction of Differentiation of UT-7/GM Cells*

UT-7/GM cells are known to differentiate into either the erythroblastic phenotype or the megakaryoblastic phenotype when they are cultured in the presence of erythropoietin or thrombopoietin, respectively (13). As shown in Fig. 4, the expression of ST2V dramatically decreased in the course of differentiation of UT-7/GM cells. The phenomenon was reproducible in two independent experiments and maintained even two weeks after the initiation of differentiation (Fig. 4B).

To clarify the physiological meaning of the expression of ST2V mRNA in UT-7/GM cells together with other cell lines, detection and determination of localization of the native ST2V protein is extremely important. Further studies are in progress using monoclonal antibodies against human ST2 protein.

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