J. Biochem. 124, 540-546 (1998)

Multiple Novel Transcripts for Apolipoprotein(a)-Related Gene II Generated by Alternative Splicing in Tissue- and Cell Type-Specific Manners[1]

Noriaki Takabatake, Masayoshi Souri, and Akitada Ichinose[2]

Department of Molecular Pathological Biochemistry, Yamagata University School of Medicine, Iida-Nishi 2-2-2, Yamagata 990-9585

Received for publication, March 19, 1998

Various Kringle 4 (K4) sequences were identified in human genomic clones and genomic DNAs amplified by PCR. These K4s were homologous to those found in the apo(a) gene and thus termed apo(a)-related genes (ARGs). The same sequences were obtained when human peripheral leukocytes were analyzed by reverse transcription-PCR in order to study the expression mode of the ARGs. It was of note that multiple transcripts with three optional exons for an ARG (ARGII) were detected in leukocytes, indicating that they were generated by alternative splicing. All these transcripts possessed the first half of the second K4 sequence, which had been reported to be skipped. The variant products of ARGII are expected to contain an additional region of either 44, 66, or 100 unique amino acids at the C-terminus of a single K4 unit. When normal human tissues and cultured tumor cells were analyzed, the multiple ARGII transcripts were detected at varying levels. The presence of the cellular state-specific alternative splicing machinery may provide not only redundancy but also diversity in the structure/function of ARGII.

Key words: alternative splicing, apolipoprotein(a), Kringle domain, plasminogen, reverse transcription-PCR.

Plasminogen is a key proenzyme of plasmin in the fibrinolytic and thrombolytic systems, deficiency of which leads to a mild thrombotic tendency (*1, 2*). Apolipoprotein(a) [apo(a)] is a protein component of lipoprotein(a), high plasma levels of which constitute a risk factor for atherosclerotic thromboembolic disease (*3, 4*). Plasmin also appears to play an important role in processes involving the breakdown of extracellular matrices, such as tumor cell migration, angiogenesis, and tissue remodeling (*5*).

During the course of studies to characterize normal and abnormal plasminogen genes (*6-8*) and the gene for apolipoprotein(a) (*9-11*), we identified many new genes homologous to those for plasminogen and apo(a), including plasminogen-related genes A, B, and C (PRGA, PRGB, and PRGC in Refs. *9* and *12*), and apo(a)-related gene [ARG; termed ARGII in this study, which was formerly designated as apo(a)II in Refs. *9* and *10*]. Furthermore, a new member of this plasminogen-apo(a) gene family has been reported (*13*). These homologues were originally thought to be pseudogenes (*6*). It has been reported, however, that PRGB is expressed in the liver, and most prominently in cancer cells (*14*), and that ARGII [referred to as apo(a)rg-C in Ref. *15*] is also transcribed in the liver (*13, 15*). Two types of complete cDNA for ARGII containing different 3′-noncoding regions have been reported by Byrne *et al.* (*15*). The liver transcript of ARGII encodes a polypeptide of 132 amino acids composed of a signal peptide and a single Kringle 4 (K4) domain. It is also known that the K4 of ARGII is encoded by two exons, and an intron is inserted at a position identical to that found in the apo(a) gene (*10*). A potential exon coding for the first half of a K4 domain was reported to be skipped because of a loss of the splicing donor site (*15*). Apart from the presence of transcripts of these homologous genes, the functions of their possible gene products remain unknown.

To understand the mode of expression of each member of this gene family, we examined transcript levels for ARGII in various normal and tumor tissues, and transformed cells in the present study. We found that multiple products of this gene generated by alternative splicing were expressed at widely varying levels. Here, we demonstrate the presence of the first half of the second K4 sequence in the novel ARGII transcripts, which was previously reported to be absent (*15*).

MATERIALS AND METHODS

Venous blood was drawn from normal individuals and tissues came from patients with cancer after informed

[1] This study was supported in part by research grants from Yamagata University, the Japanese Ministry of Education, Science, Sports and Culture of Japan (05454327), Yamagata Prefecture, Inamori Foundation, and Chiyoda Health Foundation. This paper was presented at the XIIIth International Congress on Fibrinolysis Meeting in Barcelona (1996).

[2] To whom correspondence should be addressed. Tel: +81-023-628-5275, Fax: +81-023-628-5280, E-mail: aichinos@med.id.yamagata-u.ac.jp

Abbreviations: Apo(a), apolipoprotein(a); ARG, apo(a)-related gene; K4, Kringle 4; K4a, the first half of K4; K4b, the second half of K4; RT, reverse transcription.

consent had been obtained. Genomic DNA samples were prepared from leukocytes by standard techniques.

Sequence Analysis—Genomic clones containing ARGs were obtained by screening the human libraries in the previous studies as reported in Refs. *9* and *10*. Genomic DNA inserts were excised from the phage clones with *Eco*RI and/or *Hin*dIII. The excised phage inserts and genomic DNAs amplified by PCR employing apo(a) primers were subcloned into M13mp18 and sequenced by the dideoxy termination method employing [^{35}S]dATP and buffer gradient gels. The DNA sequences of RT-PCR products and amplified genomic DNAs were also obtained using the dideoxy termination method with an ABI sequence analyzer 373A (Perkin-Elmer). Sequence homology searches for the DNA fragments were performed using the BLAST Network Service in GenBank.

RT and PCR Analyses—Total RNA was extracted by the standard guanidinium thiocyanate method from leukocytes and various tissues, followed by ultracentrifugation on a cesium chloride gradient. Reverse transcription of the total RNA (10 μg) was carried out using random hexanucleotides or an oligo dT (dT_{18}) primer and Superscript II RNase H$^-$ reverse transcriptase (GIBCO-BRL, Gathersburg, MD). The synthesized first-strand cDNA was used for PCR in a reaction mixture of 50 μl by employing various pairs of primers: for ARGII (Fig. 1; Fig. 2, A and D), 5′-GGGATTGGGACACACTTTCTGGACAC-3′ (ARGII-5, sense), 5′-TGTGAATTCTGGTTTGGAAGGATCCTCTAG-3′ (B1-3, antisense), 5′-ACAGAATTCGACAGAGCCAAGGCCTTCTGTT-3′ (B2-5, sense), 5′-TGTGAATTCTTGTATGAATGAATCCTCTAGGCT-3′ (B2-3, antisense), 5′-CAAGAATTCACTGATTGATCCAAGGCCTTCAA-3′ (B3-5, sense), 5′-AATGAATTCTTGTTTGGAAGTATCCTCTCGTC-3′ (B3-3, antisense); 5′-TCCCAGAGTGGCTGCCACCAGTA-3′ (X-F, sense), 5′-CTCTAGGATGCATCCCT-3′ (X-R, antisense), 5′-AGAGATGGATTGGCCAAAGG-3′ (Y-F, sense); for apo(a) K4A, 5′-CACGAATTCAGCAAAGGCCTGGGGTGC-3′ (sense) and 5′-CTTGAATTCAAGGAGCCTCTAGGCTTG-3′ (antisense); for both ARGII and apo(a) gene (Fig. 1; Fig. 2, A and D), 5′-CCCGAATTCAGGTGGGAGTACTGCAACCTGAC-3′ (ball-S, sense), 5′-GCGGAATTCGTGTCATAGATGACCAAGCTTGGC-3′ (a132-AS, antisense); for apo(a) gene (Fig. 1), 5′-CTGCAAGCTTCCAGATGCTGTGGCAGCTCCTTATTG-3′ (K4b-5, sense), 5′-AGCCAAGCTTTGGGTAGTATTCTGGGGTCCGACTATG-3′ (K4a-3, antisense); for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control, 5′-CATCACCATCTTCCAGGAGC-3′ (sense) and 5′-TAAGCAGTTGGTGGTGCAGG-3′ (antisense). After 25 cycles (for GAPDH) or 30-40 cycles [for ARGII and the apo(a) gene], 10 μl of the reaction mixture was applied to a 1.5 or 2.0% agarose gel. When appropriate, the gel was subjected to quantitation of the amplified products by use of a FLA-2000 Fluoroimage Analyzer (Fuji Photo Film). The fluorescence intensity of the PCR product for ARGII was normalized to that for GAPDH. Under the experimental condition used, a linear relationship was observed between the amounts of total RNA employed and the fluorescence intensity of these bands. When a sample extracted from the liver (most abundant in ARGII transcripts) was used, the relative intensity was about 400 arbitrary unit and those of all samples were within this linear range.

Cell Culture—All human cell lines were obtained from the Japanese Cancer Research Resources Bank, and A172 (glioblastoma) as well as A549 (alveolar carcinoma), C32TG (amelanotic melanoma), HeLa AG (cervical carcinoma), HepG2 and Huh7 (hepatoma), HL60 (acute promyelocytic leukemia), NEC8 (testicular germ cell tumor), SCH (gastric cancer), and U937 (monocytic leukemia) cells were maintained either in Dulbecco's modified Eagle's medium, RPMI Medium 1640, or Eagle's minimal essential medium (Nikken Biomedical Laboratory), supplemented with 10% fetal bovine serum (SEBAK GmbH), 50 μg/ml penicillin, 50 μg/ml streptomycin, and 100 μg/ml neomycin (PSN Antibiotic Mixture, GIBCO-BRL).

RESULTS

Identification of Novel K4 Sequences—In an attempt to detect an apo(a) transcript in peripheral leukocytes by RT-PCR, we synthesized a first-strand cDNA using random hexanucleotides. The cDNA product was used in the subsequent PCR employing universal apo(a)K4 primers (ball-S and a132-AS in Fig. 1, K4b and K4a, respectively), which were designed from the highly conserved regions among 11 types of apo(a) K4 (K4-1, -A, -B, -30, -31, -32, -33, -34, -35, -36, -37; Refs. *10* and *16*). As a result, a DNA band of about 240 bp was obtained (data not shown). Sequencing analysis confirmed that the band consisted of three unique fragments (designated B-I, B-II, and B-III in Fig. 2A), and that 5′-parts of these fragments coded for the second half of a K4 domain [termed K4b as defined in the genes for plasminogen and apo(a) (*6, 9, 10*)], while their 3′-parts encoded the first half (termed K4a). The transcript for plasminogen was not obtained since the two primers employed were apo(a) gene- (and ARGII-) specific. The apo(a) transcript was also not detected in leukocytes, as described later. The same three fragments were also obtained by employing an oligo(dT) primer for generation of a first-strand cDNA.

New PCR primers [ARGII-5, B1-3, B2-5 and -3, B3-5 and -3] were designed from the DNA sequences of the cDNA and exon I in ARGII [Apo(a)II in Refs. *9* and *10*], and three different types of complete K4 units of about 350 bp were amplified (PCR1, 2, and 3 in Fig. 2A; B3-5 and B1-3 in Fig. 3A). These three K4s corresponded to regions 1a and 1b, 2a and 2b, and 3a and 3b in Fig. 1. Sequencing analysis revealed that these K4s were most homologous to one of the 11 types of apo(a) K4 mentioned above, the A type [K4A (K4aA+K4bA in Fig. 1), Refs. *9* and *16*]. Moreover, three K4s were 85-90% identical in nucleotide sequence with respect to K4A. The nucleotide sequence of five cDNA fragments (PCR1-5 in Fig. 2A) generated by a combination of the primers revealed that all these K4s were connected in the order shown in Fig. 2A.

PCR using the specific primers for the three K4s and human genomic DNA as a template yielded DNA products of approximately 2.3, 4.5, and 9 kb, respectively (pcr2-4 in Fig. 2D; Fig. 3B), which products contained the sequences corresponding to the cDNA fragments. Thus, it was confirmed that the genomic regions coding for the unique K4s were, in fact, present in the human genome. These K4 sequences were also obtained by analyzing eight excised phage inserts (4H, 22E, 51E, 42E, 6H, 5E, 6HE, 4E in Fig. 1) and nine genomic DNAs amplified by PCR (a37′, a0, b1H, a1′, aX′, 367MAS, b36′, b1H, b1H′ in Fig. 1) in the

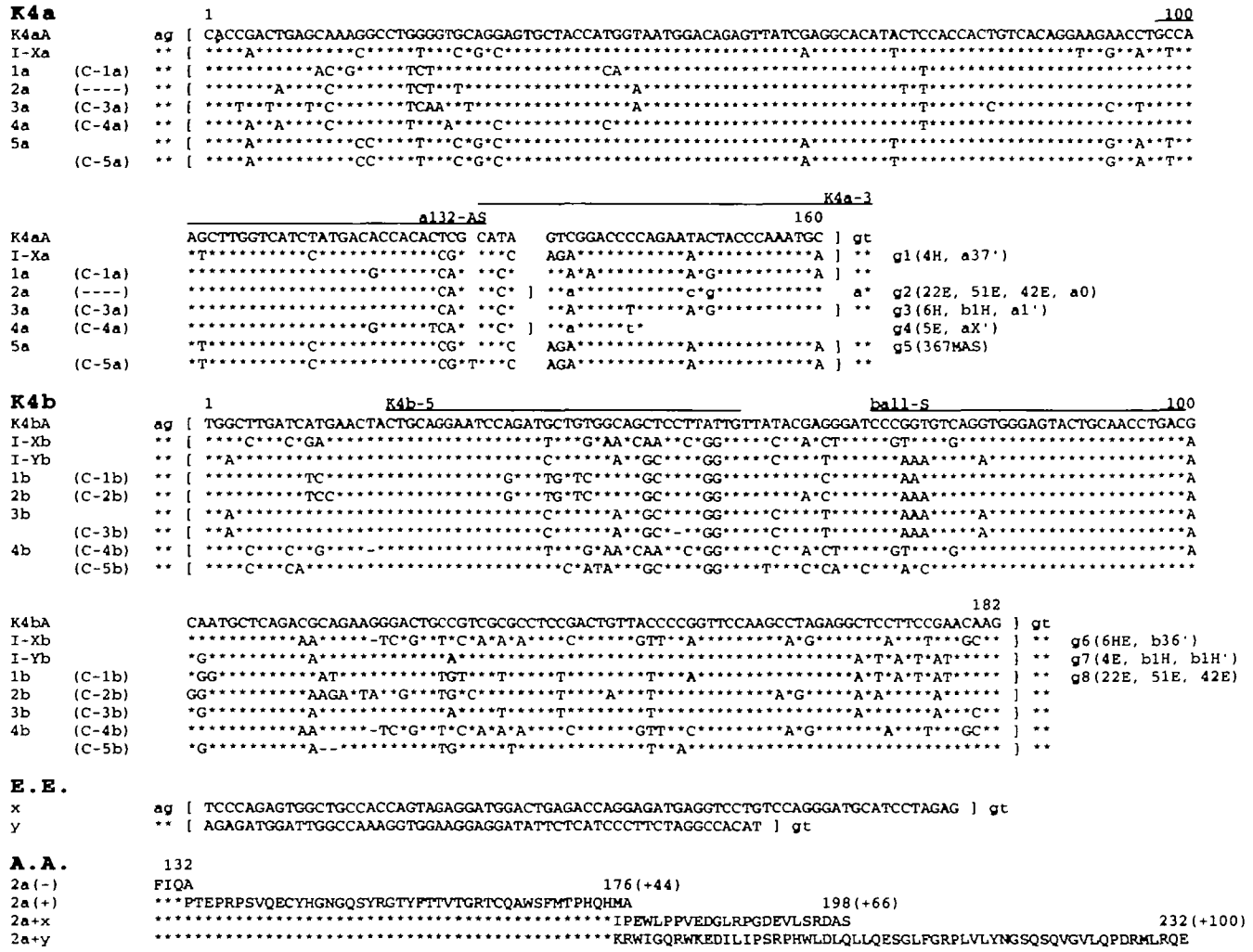

Fig. 1. **Nucleotide sequence of the first and second halves of K4 (K4a and K4b) of the gene and cDNA for ARGII, and amino acid sequence of its putative C-terminal segment (A.A.) encoded by alternative exons (E.E.).** Nucleotides in exons are shown in capitals and those in introns in lower case. The 5′ and 3′ ends of each exon are enclosed in brackets. Nucleotides identical to those of type A K4 of the apo(a) gene (K4A) are shown by asterisks. The sequences used for the preparation of universal K4 primers and apo(a)-specific primers are overlined and labeled with their names at their 5′ ends. The nucleotide sequence for ARGII including from 1a to 5a, and x and y regions were obtained in the present study by amplification of cDNA and genomic DNA, while the nucleotide sequence for ARG-C was taken from Ref. *15*. Eight DNA fragments of phage clones and nine amplified genomic DNAs obtained in the previous studies are shown in parentheses at the ends of eight genomic sequences (g1-8). I- and C- stand for ARGI and ARG-C, respectively, and -a and -b denote the first and second halves of K4, respectively. For example, 1a and C-1a refer to the first halves of the first K4 of ARGII and ARG-C, respectively, and 1b and C-1b to their second halves, respectively.

previous studies (*9, 10*). We named this novel gene ARGII, since five (g2-5, g8 in Fig. 1 at the ends of sequences) out of eight genomic K4 sequences (g1-8 in Fig. 1) completely matched those of the new K4s, while the remaining three K4 sequences (g1, g6, g7 in Fig. 1) obtained from three clones (4H, 6HE, 4E in Fig. 1 in parentheses at the ends of sequences) and four amplified DNA fragments (a37′, b36′, b1H, b1H′) did not; these K4 sequences were grouped as ARGI.

Differing In Vivo Expression of ARGII and Apo(a) in Various Normal Tissues—To see where ARGII was expressed *in vivo*, we screened a number of tissues and organs by RT-PCR using ARGII-specific primers (PCR3, Fig. 2A). DNA bands were observed for various tissues/organs (brain, eyeball, tonsil, thyroid, lung, heart, liver, fetal liver, ovary, uterus, testis, and skeletal muscle) in addition to leukocytes (selected results shown in Fig. 4B), while no band was detected for eight tissues/organs (thymus, arterial wall, pancreas, stomach, colon, spleen, kidney, and placenta), mainly of the digestive tract. Specific amplification of K4s of ARGII was confirmed by RFLP analysis of PCR products with *Hph*I, *Nco*I, *Fok*I, and *Hha*I endonucleases (data not shown); thus, the unique digestion patterns excluded any possible co-amplification of the apo(a) gene. A 253-bp fragment for GAPDH, an internal control, was obtained by RT-PCR from all tissues examined (Fig. 4C).

To quantify the ARGII transcript more precisely, the fluorescence intensity for the ARGII band was measured. Under experimental conditions, a linear relationship was observed between the amounts of total RNA employed and the fluorescence intensity of these bands (data not shown).

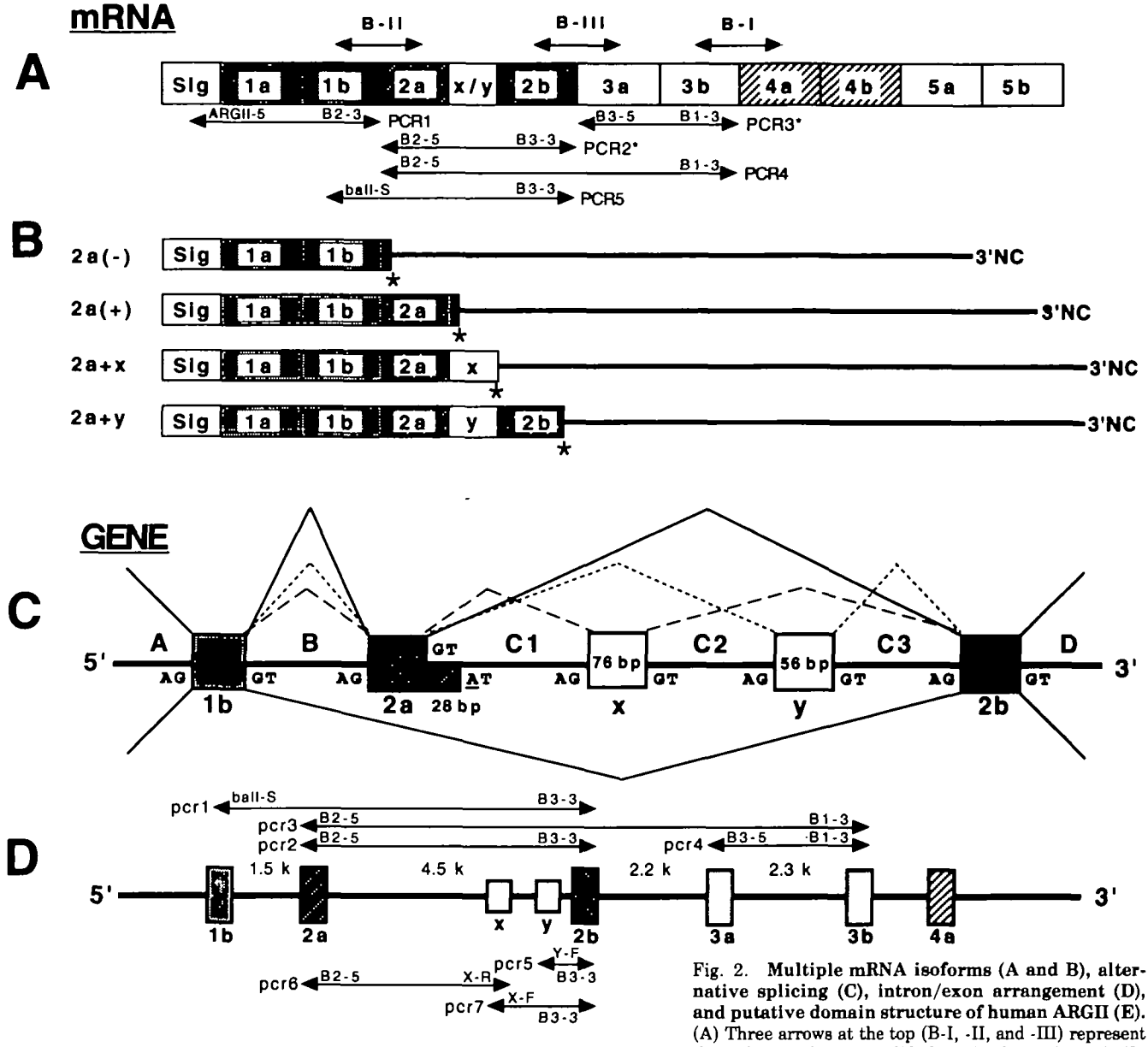

Fig. 2. **Multiple mRNA isoforms (A and B), alternative splicing (C), intron/exon arrangement (D), and putative domain structure of human ARGII (E).** (A) Three arrows at the top (B-I, -II, and -III) represent the PCR products amplified with the universal K4 primers (ball-S and a132-AS). Arrows at the bottom (PCR1-5) indicate products amplified with indicated primers [apo(a)-5, B1-5, B2-5 and -3, B3-5 and -3]. Two PCR fragments (PCR2 and 3) used for the semi-quantitation of ARGII cDNA are indicated by arrows with asterisks. (B) "sig" and "3'-NC" stand for the signal peptide for secretion and the 3'-noncoding region, respectively. Asterisks denote positions of stop codons in ARGII cDNA. (C) The exons are shown with boxes, while the introns are depicted by capitals. 2a, x, and y are alternative exons. Several lines represent alternative splicing pathways to produce multiple mRNA forms. (D) Genomic DNAs (pcr1-7) are amplified with indicated primers by arrows for confirmation of the gene structure and nucleotide sequence for ARGII. (E) Putative structures of ARGII [2a(+)] and its three variants are shown. Total numbers of amino acid residues are given at the end of each peptide.

The relative ratio of ARGII/GAPDH was then calculated. Among the six selected organs (Fig. 4, B and C), liver expressed ARGII most (2.02), followed by testis (1.41), leukocytes (0.85), lung (0.39), brain (0.09), and eyeball (0.06).

In contrast to ARGII, a discrete DNA band of about 340 bp (and 680 bp; see Fig. 5 and its legend) was detected by RT-PCR employing apo(a)-specific primers (K4b-5 and

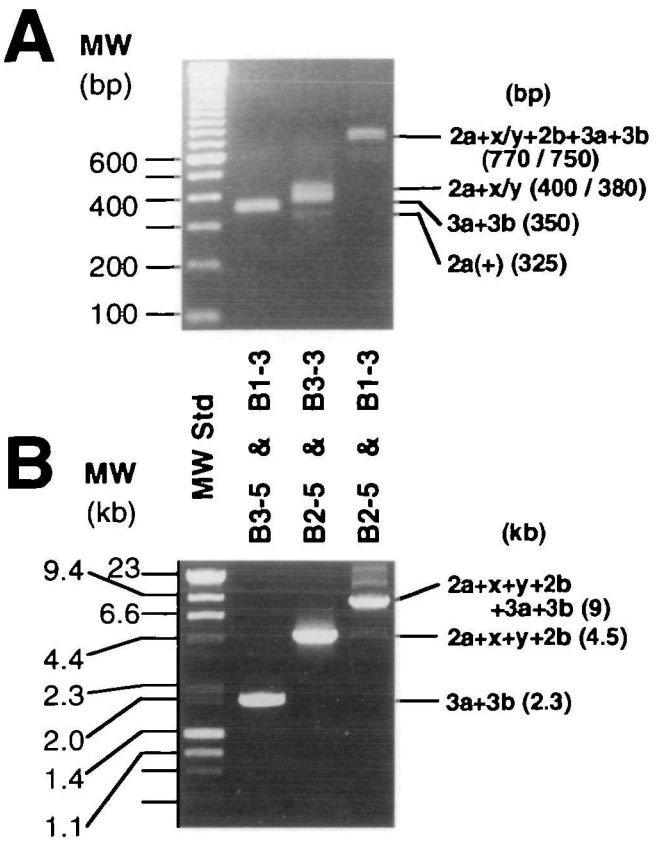

Fig. 3. **Detection of multiple ARGII transcripts in leukocytes and the corresponding regions in genomic DNA.** (A) Total RNA samples were extracted from leukocytes. Three pairs of primers, B3-5 and B1-3, B2-5 and B3-3, and B2-5 and B1-3, were employed for PCR3, 2, and 4 in Fig. 2A, respectively. (B) Genomic DNA was also used for PCR amplification.

K4a-3) in liver, while no band for apo(a) was obtained from leukocytes (Fig. 5A) or thyroid (data not shown). A faint or discrete band for an apo(a) transcript was observed for all other organs and tissues including brain, eyeball, tonsil, thyroid, thymus, lung, heart, arterial wall, fetal liver, pancreas, stomach, colon, spleen, kidney, ovary, uterus, placenta, testis, and skeletal muscle.

Expression of ARGII and apo(a) in Transformed Cell Lines—We also screened for the transcripts of ARGII and the apo(a) gene in various transformed cell lines by RT-PCR employing the same conditions used to examine normal tissues and organs. ARGII transcripts were detected in HepG2, A549, HL60, U937, and NEC8, but not in A172, Huh7, SCH, C32TG, or HeLa AG (Fig. 4B). In contrast, an apo(a) transcript was observed in HepG2, Huh7, A549, NEC8, SCH, C32TG, and HeLa AG, while no band for apo(a) was obtained from A172 or HL60 (selected results shown in Fig. 5). Thus, both the ARGII and apo(a) transcripts were found in HepG2, which is consistent with the fact that the transcripts of these genes were detected in liver as described above. When the ARGII transcript was measured more quantitatively in selected cell lines (Fig. 4, B and C), A549 contained a considerable amount of the gene product (ARGII/GAPDH ratio, 0.24), while the ratios of four other cell lines were 0.08-0.03.

Presence of Multiple Transcripts for ARGII—To detect the 2a and 2b regions of ARGII in a cDNA sample prepared from leukocytes, the 2a- and 2b-specific primers (B2-5 and B3-3, respectively) were employed in PCR. An extremely faint DNA band amplified (PCR2 in Fig. 2A; B2-5 and B3-3 in Fig. 3A) was about 325 bp in size for a single K4 unit, the sequence of which was identical to the cDNA sequence for the expected 2a and 2b regions in ARGII. In contrast, the size of a major doublet (2a+x or y in Fig. 2A, B2-5 and B3-3 in Fig. 3A) was about 380 bp, apparently larger than expected for a single K4 unit. Sequencing analysis revealed that each of the two cDNA fragments had a nucleotide insertion of either 76 or 56 bp (x or y, Fig. 1, E.E.). The sequences of additional exons x and y were also identified in genomic DNAs amplified by PCR employing the same 2a-specific primers and new x or y-specific primer (X-F, -R, Y-F, or -R), and human genomic DNA (pcr5-7, Fig. 2D). When the universal K4 primer (ball-S) and the B3-3 primer were used (PCR5 in Fig. 2A), a band of about 300 bp in size was obtained (data not shown), the sequence of which was identical to the known cDNA for the 1b and 2b regions without 2a (*15*). Accordingly, there were at least four different forms of the ARGII transcripts in leukocytes (Fig. 2B).

To determine whether the amounts of multiple forms of the ARGII transcripts with exon 2a [2a(+)] differ among tissues, 2a(+)ARGII transcripts were specifically amplified with B2-5 and B3-3. Most 2a(+)ARGII transcripts in leukocytes contained an extra exon x or y, while both ARGII with and without exon y were present in similar amounts in liver, lung, and testis (Fig. 4A). 2a(+)ARGII transcripts were also specifically amplified in the transformed cell lines that were found to express ARGII as described above. All five transformed cells contained the ARGII transcript with exon y almost exclusively (Fig. 4A). In particular, ARGII transcripts without exon y could not be detected in HepG2, U937, and HL60 cells.

DISCUSSION

When we characterized the gene for human plasminogen and its related genes and the gene for apo(a) and its related genes by genomic cloning and PCR employing genomic DNAs (*6-12*), many new genomic DNAs apparently coding for Kringle units were identified (Fig. 1). These sequences were similar to but distinct from those of apo(a) as well as those of other known genes. Therefore, we named them apo(a)-related genes (ARGs). Recently, Byrne *et al.* (*15*) independently reported the cDNA sequence for apo(a)-related gene C, "apo(a)rg-C" [referred to as apo(a)-related gene C, (ARG-C), in this paper]. Surprisingly, most portions of ARG-C were identical to the corresponding regions of ARGII, except for the absence of a C nucleotide in C-3b and the presence of a T nucleotide in C-5a of "ARG-C" (Fig. 1). Our data suggest that these differences are due to sequencing errors, since these nucleotides were present and absent, respectively, in all of more than 10 amplified genomic DNAs for the 3b and 5a regions obtained in our study. ARGII was assigned to chromosome 6 by PCR of genomic DNAs from two panels of human/hamster hybridomas (Takabatake and Ichinose, unpublished data), where the apo(a) gene and ARG-C were localized side by side (*17*). Moreover, an "apo(a)-like gene" is claimed to be identical to ARGII (*13*), although the authors of this claim

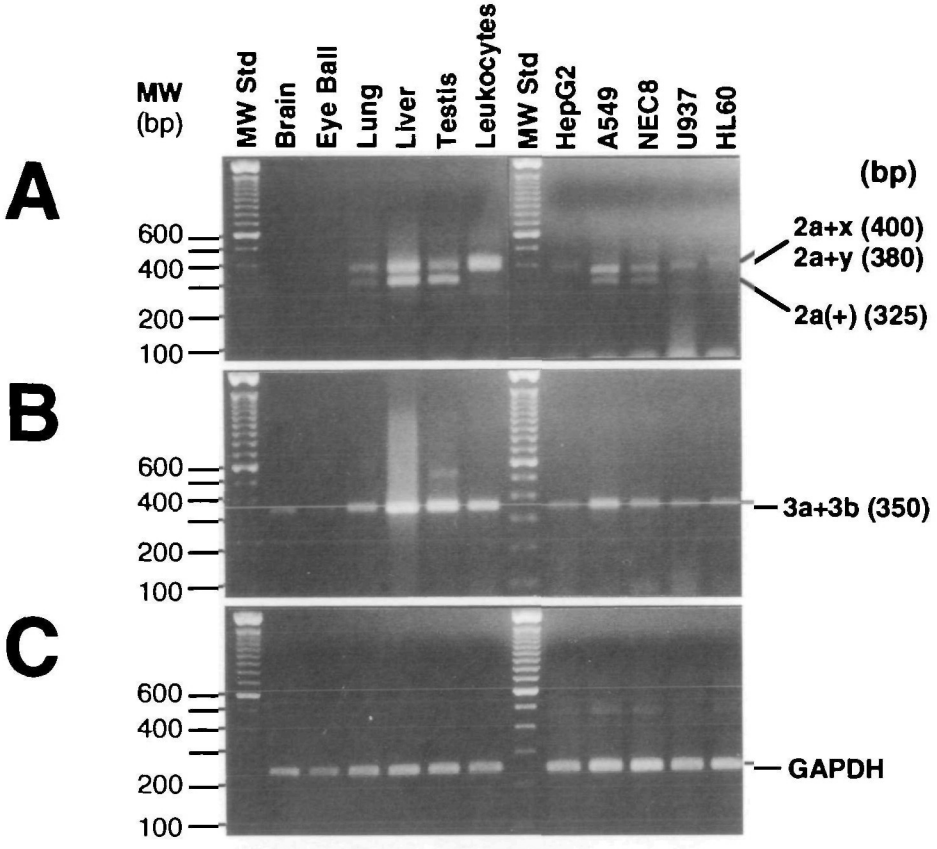

Fig. 4. **Tissue- and cell type-specific expression of multiple ARGII transcripts.** Total RNA samples were extracted from leukocytes, normal tissues, and cultured transformed cells. The primers B2-5 and B3-3 were employed for PCR2 in Fig. 2A (A) and B3-5 and B1-3 for PCR3 (B). GAPDH transcript was also amplified as an internal control (C). The amount of PCR product for ARGII was normalized to that of GAPDH to estimate the relative level of ARGII expression in each sample.

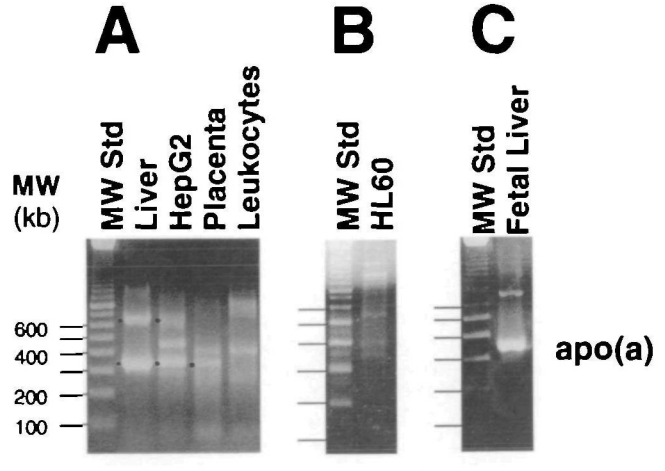

Fig. 5. **Apo(a) transcript in various tissues and cell types.** Total RNA samples were extracted from normal tissues, and cultured transformed cells including liver, HepG2, placenta, and leukocytes (A), HL60 (B), and fetal liver (C). The primers, K4b-5 and K4a-3, were employed for amplification. Bands of about 340 and 680 bp correspond to apo(a) transcripts due to the presence of repetitive K4A units in the apo(a) gene. Other bands were non-specific PCR products, the sequences of which were determined by the dideoxy termination method.

do not present the nucleotide sequence. Thus, it was concluded that ARGII and ARG-C were the same gene. We retain our nomenclature since "ARG-B" of Byrne's terminology is in truth a plasminogen-related gene (*13*) rather than an ARG. Furthermore, there is no "ARG-A" in Byrne's naming system.

In contrast to ARGII, an apo(a) transcript was detected by RT-PCR in most tissues/organs though not in thyroid or leukocytes, suggesting that the expression of these two homologous genes is controlled by different mechanisms. This hypothesis is consistent with a fact that the nucleotide sequence more than 160 bp upstream in the 5′-flanking region of ARGII is quite different from that in the apo(a) gene (*10*). Accordingly, there may be different enhancers and silencers in the two homologous genes.

It was of particular interest that the 2a region was either present or absent in the ARGII transcripts (Figs. 1 and 2), while it was not detected in the "ARG-C" transcript (*15*). The sequence of this portion in our cDNA exactly matched the potential exon of an amplified genomic DNA and fragments of three genomic phage clones (a0 and 22E, 51E, and 42E, Fig. 1). In the genomic DNA sequences, there was an AT dinucleotide rather than the consensus GT dinucleotide at the potential splicing donor site (Fig. 1, K4a), which resulted in "exon skipping" (*15*).

The lack of the splicing donor site also led to an alternative splicing, since a cryptic splicing site 28 bp upstream in exon 2a (coding for the 2a region) was utilized. The sequences of the additional exons x and y indicate that the two cDNAs were generated by mutually exclusive alternative splicing (Fig. 2C). Accordingly, potential translated products of ARGII would contain an additional region of either 44, 66, or 100 amino acids (Fig. 1, A.A.) when compared to a product lacking exon 2a [2a(−)]. The alternative splicing modifies the C-terminus of the putative ARGII protein. Computer searches produced no evidence of

sequences homologous to the two unique C-terminal segments encoded by exons x and y.

It was noteworthy that most 2a(+)ARGII transcripts in leukocytes contained an extra exon x or y, while both ARGII with and without y were present in similar amounts in liver, lung, and testis. These results suggested the presence of cellular state-specific alternative splicing machinery (*18*), which may provide not only redundancy but also diversity in the structure/function of ARGII. It was of interest that transformed cell lines including HepG2, U937, and HL60 cells contained the ARGII transcript with exon y exclusively. These results indicate the presence or absence of a trans-acting factor(s) for splicing, which is relatively specific among these transformed cells.

The production of mRNA isoforms by alternative splicing is a rather widespread phenomenon observed in various cancers (*18*). The patho-physiological function of ARGII, however, remains unknown. The K4 domain of apo(a) plays an essential role in its fibrin binding and in inhibition of the fibrin binding of plasminogen, which results in the inhibition of plasmin generation (*3, 4*). Accordingly, increased levels of the gene product of ARGII composed of the K4 domain alone or with additional peptides at the C-terminus may affect the activation of plasminogen to plasmin, and may play a role in the invasion and metastasis of cancer cells, such as extracellular matrix destruction (*5*). This assumption is analogous to the fact that a 38-kDa fragment of plasminogen (K1-3) named angiostatin potently inhibits neovascularization, growth of tumors and their metastases (*19*).

In the present study, 5 out of 10 transformed cell lines were found to contain the ARGII transcript. Similarly, it has been reported that the ARGII transcript was detected in six out of 13 breast tumor tissues (*15*). Furthermore, our preliminary study demonstrated that ARGII was expressed in one of the primary lung cancer tissues, but the amount was similar to normal lung tissue obtained from the same patient (Souri and Ichinose, unpublished data). This primary lung cancer was classified as stage IIIB lung carcinoma, and diagnosed as small cell carcinoma by histological examination. The cancer tissue contained about four times as much 2a(+) transcript as the normal tissue, suggesting that the increased expression of exon 2a(+) mRNA may be related to malignant transformation. Five other primary lung carcinoma tissues, all of which were squamous cell carcinoma, showed no apparent increase in the amplified 2a(+)ARGII band. Thus, the expression of 2a(+)ARGII may be induced specifically in small cell carcinoma. Further studies of adequate sample size will be required to confirm these findings.

The authors thank Drs. T. Hashiguchi, S. Tsutsumi, T. Izumi, K. Fujimaki, and K. Suzuki for their helpful discussion, and L. Boba for her help in the preparation of the manuscript.

REFERENCES

1. Robbins, K.C. (1992) Dysplasminogenemia. *Prog. Cardiovasc. Dis.* **34**, 295-308
2. Girolami, A., Sartori, M.T., Saggiorato, G., Sgarabotto, D., and Patrassi, G.M. (1994) Symptomatic versus asymptomatic patients in congenital hypoplasminogenemia: a statistical analysis. *Haematology* **26**, 59-65
3. Utermann, G. (1989) The mysteries of lipoprotein(a). *Science* **246**, 904-910
4. Miles, L.A. and Plow, E.F. (1990) An interloper into the fibrinolytic system? *Thromb. Haemost.* **63**, 331-335
5. Saksela, O. and Rifkin, D.B. (1988) Cell-associated plasminogen activation: regulation and physiological functions. *Annu. Rev. Cell Biol.* **4**, 93-126
6. Petersen, T.E., Martzen, M.R., Ichinose, A., and Davie, E.W. (1990) Characterization of the gene for human plasminogen, a key proenzyme in the fibrinolytic system. *J. Biol. Chem.* **265**, 6104-6111
7. Ichinose, A., Espling, E.S., Takamatsu, J., Saito, H., Shinmyozu, K., Maruyama, I., Petersen, T.E., and Davie, E.W. (1991) Two types of abnormal genes for plasminogen in families with a predisposition for thrombosis. *Proc. Natl. Acad. Sci. USA* **88**, 115-119
8. Tsutsumi, S., Saito, T., Sakata, T., Miyata, T., and Ichinose, A. (1996) Genetic diagnosis of dysplasminogenemia: Detection of an Ala601-Thr Mutation in 118 out of 125 families and identification of a new Asp676-Asn mutation. *Thromb. Haemost.* **76**, 135-138
9. Ichinose, A. (1992) Multiple members of plasminogen-apolipoprotein(a) gene family associated with thrombosis. *Biochemistry* **31**, 3113-3118
10. Ichinose, A. (1995) Characterization of the apolipoprotein(a) gene. *Biochem. Biophys. Res. Commun.* **209**, 365-371
11. Ichinose, A. and Kuriyama, M. (1995) Detection of polymorphisms in the 5′-flanking region of the gene for apolipoprotein(a). *Biochem. Biophys. Res. Commun.* **209**, 372-378
12. Kida, M., Wakabayashi, S., and Ichinose, A. (1997) Characterization of the 5′-flanking region of plasminogen-related genes A and B. *FEBS Lett.* **404**, 95-99
13. Magnaghi, P., Citterio, E., Malgaretti, N., Acquati, F., Ottolenghi, S., and Taramelli, R. (1994) Molecular characterization of the human apo(a)-plasminogen gene family clustered on the telomeric region of chromosome 6 (6q26-27). *Hum. Mol. Genet.* **3**, 437-442
14. Weissbach, L. and Treadwell, B.V. (1992) A plasminogen-related gene is expressed in cancer cells. *Biochem. Biophys. Res. Commun.* **186**, 1108-1114
15. Byrne, C.D., Schwartz, K., and Lawn, R.M. (1995) Loss of splice donor site at a 'skipped exon' in a gene homologous to apolipoprotein(a) leads to an mRNA encoding a protein consisting of a single Kringle domain. *Arterioscler. Thromb. Vasc. Biol.* **15**, 65-70
16. McLean, J.W., Tomlinson, J.E., Kuang, W.J., Eaton, D.L., Chen, E.Y., Fless, G.M., Scanu, A.M., and Lawn, R.M. (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* **330**, 132-137
17. Byrne, C.D., Schwartz, K., Meer, K., Cheng, J.-F., and Lawn, R. M. (1994) The human apolipoprotein(a)/plasminogen gene cluster contains a novel homologue transcribed in liver. *Arterioscler. Thromb.* **14**, 534-541
18. Norton, P.A. (1994) Alternative pre-mRNA splicing: factors involved in splice site selection. *J. Cell Sci.* **107**, 1-7
19. O'Reilly, M.S., Holmgren, L., Shing, Y., Chen, C., Rosenthal, R.A., Moses, M., Lane, W.S., Cao, Y., Sage, E.H., and Folkman, J. (1994) Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. *Cell* **79**, 315-328