Identification and Cloning of a New Gene (2A3-2), Homologous to Human Translational Elongation Factor, Upregulated in a Proliferating Rat Smooth Muscle Cell Line and in Carotid Hyperplasia

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Abstract—Smooth muscle cells (SMCs), before migration and proliferation in the intima of the vessel wall, change from a normal contractile to a pathological proliferating phenotype. The molecular regulatory mechanisms implicated in such phenotypic changes remain poorly understood. In this study, using differential display, we have isolated for the first time a new gene (2A3-2) that is overexpressed in a rapidly proliferating, but not synthetic, rat SMC line. This was further confirmed by northern blot performed on the 2 cell types. Moreover, balloon catheter injury of rat carotids showed, by a virtual northern technique, an upregulation of this new gene in hyperplasia vessels. This new gene (2A3-2, 1.2 kb) was present in skeletal muscle, heart, aorta, lung, liver, kidney, and spleen. In addition, 5' rapid amplification of cDNA ends (5' RACE) allowed the cloning and sequencing of this 1.2-kb gene. Comparison of this newly identified gene sequence with data banks showed a strong homology to human and bovine mitochondrial translational elongation factor. The 2A3-2 gene, identified in this study, may play a vital role in the cascade of events implicated in switching SMC phenotype from a quiescent to a proliferate one. 

Key Words: smooth muscle cell ■ differential display ■ virtual northern ■ rat carotid hyperplasia ■ cell culture ■ translational elongation factor

Arterial smooth muscle cells (SMCs) are a major component of atherosclerotic and restenotic plaques. Migration and proliferation of these cells is known to play a key role in lesion formation and atherogenesis. During the initial stages of these pathological processes, arterial SMCs migrate into the intima, change the ratio of myofilaments to the endoplasmic reticulum and Golgi apparatus, proliferate, and produce extracellular matrix components. Subsequently, SMCs are transformed into foam cells by accumulating lipids. In these pathologies, SMCs undergo a phenotypic modulation where they change from a contractile to a synthetic and then to a proliferating state. Harvested SMCs, during cell culture passages, progressively lose their contractile phenotype and exhibit characteristics identical to those observed in synthetic SMCs of diffuse intimal thickening. In long-term cultures, certain rat aortic SMCs were shown to generate a transformed phenotype with several similarities to highly proliferating cells. Little is known at this stage about the cascade of genes implicated in changes of SMC phenotype.

Proliferation of SMCs in the intima of the vessel wall is thought to be either the result of an inflammatory process or that of a monoclonal growth. According to Ross, atherosclerotic lesions result from an excessive inflammatory fibroproliferative response to various forms of insult to the endothelium and SMCs of the artery. A large number of growth factors, cytokines, and vasoregulatory molecules participate in this process. After lesion formation, endothelial cells secrete chemotactic factors that would induce proliferation of SMCs and lipid deposition. LDLs that have been oxidized also participate in the formation of foam cells and atherosclerotic lesions. On the other hand, Benditt and Benditt et al hypothesized that a neoplastic process could arise during atherogenesis in response to a mutational or viral event. Indeed, certain genes have been shown to be implicated in the pathobiology of atherosclerosis and cancer. This hypothesis has been supported by experimental data demonstrating the appearance of SMCs in aortas of animals injected with carcinogens alone, in combination with methoxamine, or with oncogenic Marek’s herpes virus. Moreover, DNA from human plaques was shown to completely transform transfected NIH 3T3 cells. Other factors, such as bacterial or viral agents (herpes virus and cytomegalovirus), have been detected and may be involved in the initiation and perpetuation of atherosclerotic lesions. In the present study, gene expression in normal rat synthetic SMCs at passage 9 (P9 cells) were compared with rapidly proliferating SMCs (V8 cell line)

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by differential display. A differentially expressed gene, present at high levels in rapidly proliferating cultured SMCs, was also shown to be upregulated in balloon catheter–injured, but not in normal, carotids.

Methods

Surgical procedures and animal care conformed strictly to the Guidelines of the National Institute of Health and Medical Research (Decree No. 87-848 of October 19, 1987). Sprague–Dawley rats (species, Rattus rattus; strain, OFA, Iffla Credo, France) used in this study were anesthetized with an intraperitoneal injection of pentobarbital (0.11 mL/100 g of body weight).

Cell Culture

Primary SMCs were obtained from explants of medial thoracic aortas from 7- to 8-week-old male Sprague–Dawley (250 g) rats and cultured as previously described.14,30–32 Cell samples were preserved in liquid nitrogen at passages 2 to 10 and then every 10 passages. SMCs at passage 9 were shown to be in a synthetic state. A spontaneously highly proliferating rat SMC line17 (V8) has been used in this study. This cell line was established from aortic media of adult rat and passed for greater than 200 times.17 Both cell types were grown in MEM medium supplemented with 10% newborn calf serum. The growth rate of P200 passaged cells was shown to be higher (×1.4) than that of P9 synthetic cells.17 To reach, at the same time, a similar cell density and confluence, P9 and P200 cells were seeded respectively at 14×10^4 and 10×10^4 cells/cm^2 in 25-cm^2 Falcon flasks. These cells, confluent within 5 to 7 days, were trypsinized and aliquots counted (60×10^4 to 80×10^4 cells/cm^2). Before RNA extraction, cultures were growth-arrested by incubation in MEM medium supplemented with 0.1% newborn calf serum for 24 hours.

Total and Poly(A)+ RNA Preparation

After cell culturing, cells were washed with Hank’s medium (Sigma) and used for the RNA preparation. Total RNA was extracted by using the guanidinium thiocyanate53 method. For differential display analysis, genomic DNA contamination was removed by DNase I (MessageClean, GenHunter). For cDNA library construction and rapid amplification of 5’ cDNA ends (5’ RACE), poly(A)+ RNA was isolated from total RNA using oligo(dT)_6 primers (Oligotex mRNA kit, Qiagen).

Differential Display Analysis

Differential display was performed as previously described29 (RNAImage, GenHunter).

Reverse Transcription (RT) Reaction

Total RNA (0.2 μg) from each sample was reverse transcribed with 100 U of Moloney murine leukemia virus reverse transcriptase in the presence of 250 μmol/L dNTPs and 2 μmol/L H-T11 M (M can be either dA, dG, or dC, and H is the HindIII restriction site). The 18-μL RT reaction was reverse-transcribed for 1 hour at 37°C, then the enzyme was denatured by heating at 75°C for 5 minutes.

PCR Amplification

Single-strand cDNA mixture (2 μL) thus obtained was used for 8 different PCR reactions, each containing a different arbitrary primer from the 5’ end. The 18-μL PCR mix included 2 μmol/L of the H-T11 M primer (same as RT), 2 μmol/L of a specific arbitrary primer, 25 μmol/L of 5’ dNTPs with 0.25 μL of [ε-32P]dATP (2000 Ci/mmol, Amersham), and 1 U of Taq DNA polymerase (Perkin-Elmer). Thermal cycling amplification parameters (40 cycles), using GeneAmp PCR System 9600 (Perkin-Elmer), were as follows: 94°C (15 s), 40°C (2 minutes), 72°C (30 s), and a final 5-minute extension step at 72°C.

Separation by Electrophoresis

Only 3.5 μL of the PCR products was separated on a 6% denaturing polyacrylamide gel in tris-borate–EDTA buffer after addition of 2 μL of loading dye (95% formamide, 10 mmol/L EDTA, pH 8.0, 0.09% xylene cyanol, and 0.09% bromophenol blue). The gels were run for 4 hours at 1400 V, dried without fixation for 2 hours at 80°C, exposed for 72 hours, and then visualized by autoradiography.

Band Recovery, Cloning, and Sequencing

(1) Differentially expressed bands (upregulated or downregulated) were recovered under sterile conditions by excising the gel slice from the dried gel by using a razor blade. Each gel slice was placed in 100 μL of sterile water, boiled for 15 minutes to solubilize the DNA, and then ethanol-purified. (2) PCR-reamplification is performed with 4 μL of purified fragment, using the same primer pair and PCR parameters that gave rise to the band. (3) Reamplified DNA fragments were run on a 1.5% agarose gel. Bands that succeeded to be reamplified were cloned into PCR II vector (TA cloning kit, Invitrogen). (4) For DNA sequencing, minipreps of plasmid DNA were performed,34 followed by the dyeodeoxy sequencing method35 (T7 Sequencing Kit, Pharmacia).

Homologies With the Data Banks

The sequences obtained were compared with known sequences by searching in the different databases (eg, GenBank, EMBL, EST, and STS), using the BLAST (basic local alignment search tool)36 and FASTA37 programs.

Probes and Northern Blot

Total RNA was extracted as above, denatured, separated by electrophoresis, hybridized to a nylon membrane (Hybond, Amersham). After capillary blotting performed overnight, the membrane was baked for 2 hours at 80°C. Probes for northern blots were prepared following the random priming method (High Prime), using the PCR-amplified inserts in the PCR II vector described above, and then purified by using G-Sephadex (Quick Spin Columns, Boehringer). Prehybridization and hybridization were performed according to standard protocols.38 Blots were exposed with intensifying screens against a Kodak film for 1 week at –70°C. Similar loading of RNA was assessed by using the actin probe.

Balloon Catheter Injury of the Rat Left Carotid Artery

Carotid arteries were obtained from 10- to 14-week-old male Sprague–Dawley rats (350 g). Neointima formation was induced as previously described.39 In brief, the left carotid was exposed under a microsurgical operating microscope (OPMI 7, Carl Zeiss). After incision of the left external carotid artery, the balloon catheter (2F Fogarty, Baxter) was introduced through the primitive carotid artery. Injury of the left primitive carotid was produced by passing the inflated balloon catheter back and forth into the carotid for 3 times. The balloon was sufficiently inflated to generate slight resistance and the catheter was then removed, to ligate the external artery. Rats were kept under ad libitum conditions for 3 weeks after balloon injury, after which they were anesthetized by urethane (Sigma) for vessel extraction. Each carotid artery was cut into 2 pieces; the first was immediately snap-frozen in liquid nitrogen for RNA extraction and the second was fixed, to be used for histological and morphological controls. The right carotid, considered the normal control artery, was also extracted. This experimental model is known to induce a neointimal hyperplasia similar to that observed in humans after angioplasty.

SMART Technique and Virtual Northern Blot

Because of limited amounts of total RNA, gene expression analysis of rat carotids was performed by a tandem of SMART-PCR (Clontech) and virtual northern blot, as opposed to a standard northern blot. A full description of the SMART technology, and its applications, is given in the Clontech manual. In brief, single-strand cDNA is synthesized with 1 μg of total RNA from various samples (P9 and V8 cells, aorta, and right and treated left carotids). The RT is performed for 1 hour at 42°C, using 200 U/μL of Superscript II transcriptase (GibcoBRL), 10 μmol/L of the cDNA synthesis primer (CDS), 10 μmol/L of SMART oligo, 10 mmol/L dNTP, and 20 μmol/L DTT. When the 5’ end of the mRNA is reached, the enzyme’s terminal transferase activity adds a few nucleotides, primarily deoxyctydine, to the 3’ end of the cDNA. RT then
switches templates and continues replicating to the end of the oligo at the 5' mRNA end. The RT reaction was then heated at 72°C for 5 minutes. The resulting full-length single-strand cDNA contains a sequence complementary to the SMART oligo, which is then used as the template in PCR to generate double-stranded cDNA. Choosing the optimal number of PCR cycles ensures that the double-stranded cDNA will remain in the exponential phase of amplification. In our experiments, the optimal number of cycles was 16 because the plateau was reached after 17 cycles. Only single-strand cDNA having the SMART sequence at the 5' end and the oligo(dT) at the 3' end are exponentially amplified by PCR. The 100-μL PCR reaction contains 10 mmol/L of dNTP, 10 μmol/L of the PCR primer (complementary to the SMART oligo and CDS primer), 2 μL of the 50× KlenTaq polymerase, and 10 μL of the 10× KlenTaq PCR buffer. PCR conditions are as follows: 1 step at 95°C (1 minute), followed by 16 cycles of 95°C (15 s), 65°C (30 s), and 68°C (6 minutes). A virtual northern blot is obtained by first running 0.5 μg of SMART-PCR–amplified cDNA on an agarose/ethidium bromide gel. Subsequently, the electrophoresed material is transferred to a nylon membrane (Hybond N, Amersham), which is then probed with 32P-labeled 2A3-2 fragment, as in a standard northern blot. Actin is also used to assess similar loading.

5' Rapid Amplification of cDNA Ends (5' RACE)

To obtain the upstream 5' region of the new gene, the 5' RACE technique was performed basically by applying the touchdown PCR principle and by using Marathon cDNA Amplification and Advantage KlenTaq Polymerase kits (Clontech). In the first step, single-strand cDNA is synthesized with 1 μg of V8 poly(A)+ RNA, using 10 μmol/L of the cDNA synthesis primer and Moloney murine leukemia virus reverse transcriptase for 1 hour at 42°C. DNA synthesis was verified by the addition of dNTPs among which 1 was radiolabeled with [α-32P]dCTP (1 μCi/μL, NEN). The second step is the synthesis of double-stranded DNA performed at 16°C for 4 hours in an enzyme mixture containing Escherichia coli DNA polymerase I, RNase H, and E. coli DNA ligase. These enzymes allow the synthesis of double-stranded cDNA, RNA degradation, and the formation of blunt ends, respectively. A 1% agarose gel electrophoresis is performed to estimate the quantity and quality of the double-stranded cDNA synthesized. The gel is then dried and put in contact with a Kodak film at −70°C to visualize the DNA smear.

The third step allows us to obtain a library of double-stranded cDNA, from V8 cells, by ligating an adapter to both ends of the double-stranded cDNA, using a T4 DNA ligase at 16°C overnight.

In the last step, an aliquot of the library is subjected to PCR. The 50-μL PCR reaction contains 10 μmol/L of dNTP, 10 μmol/L of the adapter primer (complementary to the cDNA adapter), 5 μL of the 50× KlenTaq polymerase, and 10 μmol/L of gene specific primer (GSP) complementary to the 3' differentially expressed fragment (2A3-2 GSP: 5'GGGTAAAGTTATATATAATCACATTGAT-AAAACG-3'). The mixture was subjected to a PCR step at 94°C (1 minute) followed by 33 cycles of 94°C (30 s), 60°C (30 s), and 68°C (2 minutes 15 s), and a 5-minute extension step at 72°C. The amplified DNA fragments were cloned into the PCR II vector and purified by using Qiagen Plasmid Midi Kit (Qiagen). The insert DNA is then sequenced commercially (Genome Express).

Results

Identification and Cloning of the 2A3-2 Gene

On initial differential display gels, we observed 51 bands, using 8 arbitrary and 3 RT primers, that were differentially expressed between synthetic and rapidly proliferating cells (P9 and V8). Low molecular weight bands gave little sequence information and were often, as verified by PCR reamplification and northern-blots, found to be false positives. Of 36 high molecular weight bands retained, 22 bands were reamplified and cloned into PCR II plasmid. Sequences from different clones were then sent to databases for identity and homology search. Northern-blot was then used, for some of the newly identified cDNA fragments, to assay levels of expression in both cell types. One of the genes that was clearly differentially displayed was band 2A3-2 (Figure 1). This cDNA fragment showed an upregulation, using northern blots, in rapidly proliferating cells compared with synthetic cells (Figure 2A). Quantification of 2A3-2 signals, reported

Figure 1. A representative differential display (DD) analysis showing the 2A3-2 cDNA band. Total RNA was extracted from synthetic (P9) and proliferating (V8) SMCs, then subjected to DD. Migration of PCR products was performed on a denaturing 6% polyacrylamide gel, using arbitrary primer AP2 and a 3' primer (dT11,A). Lanes 1 and 2 correspond to synthetic and proliferating cells, respectively.

Figure 2. Northern blot analysis with the 2A3-2 cDNA band. A, The 2A3-2 gene is upregulated in proliferating (V8) but not synthetic cells (P9). Quantification of 2A3-2 signals, reported to the β-actin levels, showed a 4-fold increase in the P200 compared with the P9 cells. This increased expression of the 2A3-2 gene in P200 compared with P9 was repeatedly observed (n=3) in different northern blots. The 2A3-2 gene has a molecular weight of 1.2 kb as shown by the northern blot. Lanes 1 and 2 correspond respectively to synthetic and rapidly proliferating cells. B, Rat multiple-tissue northern-blot analysis with the 2A3-2 cDNA band. The blot contained 16 μg of total RNA from various rat tissues and was probed with the 2A3-2 cDNA fragment isolated by differential display. Sizes of RNA markers are shown on the left (in kb). Transcripts of ~1200 bp could be observed in all rat tissues analyzed. A lower abundant transcript of ~2.4 kb is observed for skeletal muscle. Lane 1, skeletal muscle; lane 2, heart; lane 3, lung; lane 4, liver; lane 5, spleen; lane 6, kidney; and lane 7, aorta.
to the actin levels, showed a 4-fold increase in the P200 compared with the P9 cells. This increased expression was repeatedly observed (n=3) in different northern blots. The molecular weight of 2A3-2, as shown by northern, was determined to be 1.2 kb.

**Tissue Distribution of 2A3-2 Gene**
A rat multiple-tissue northern blot, probed by the 2A3-2 cDNA band, showed the 1.2-kb gene to be present in skeletal muscle, heart, aorta, lung, liver, kidney, and spleen tissues (Figure 2B). Skeletal muscle contains not only the normal 1.2-kb mRNA, but also a lower abundant transcript of ~2.4 kb. This transcript may arise from the use of an alternative polyadenylation site. The multiple northern blot shows that the 2A3-2 gene is not an artifact induced by cell culturing but is present in different tissues.

**SMART and Virtual Northern of Carotids**
Neointimal hyperplasia of rat carotids, induced by balloon catheter injury, was used to investigate the in vivo role of this new gene in SMCs. Rat left carotids were treated by a balloon catheter to initiate SMC proliferation and neointima formation. In contrast, right untreated carotids were used as controls (Figure 3). Because the amount of total RNA extracted from 1 carotid was <2 μg, we used a tandem of SMART-PCR and virtual northern blot to assay expression levels of 2A3-2. This tandem approach of technologies gives information similar to that obtained by a standard northern blot. The 2A3-2 gene was shown to be upregulated (4-fold increase) in the left balloon-treated carotid, in comparison with the control right carotid. The virtual northern experiment was repeated and confirmed on 5 different blots. Virtual northern blots further confirmed 2A3-2 upregulation in V8, but not in P9, cells as previously observed by standard northern (Figure 4A). It is noteworthy that the expression of 2A3-2 in P9 cells, under quiescent (48 and 72 hours) or proliferating conditions (0, 4, 8, and 24 hours), was not altered by the state of the proliferation of the cells (Figure 4C).

**5' RACE and Sequencing of the 2A3-2 Gene**
The full length of this gene was obtained by 5' RACE, using a cDNA library constructed from rapidly proliferating V8 cells. The molecular weight of the 5' RACE product was 1.2 kb as previously indicated by northern blot (Figure 5). The PCR product was amplified, purified, cloned, and sequenced (Figure 6A). The full 2A3-2 nucleotide sequence was then sent to the European Molecular Biology Laboratory (EMBL) database, using Sequin software, to get an accession number (AJ006151).

**Characteristics of the 2A3-2 Gene**
The open reading frame of the sequenced gene (1149 bp) was identified and shown to contain 972 bp running from the initiation codon (designated +1) to a TAG stop codon (Figure 6). This gene had a very short 5' untranslated region (7 bp long). The initiation codon (designated +1) is preceded by a G residue at position −3 and followed by a T residue at position +4. Analysis of numerous translational start sites shows that the consensus sequence has a purine at position −3 and a G residue at position +4. Finally, the cDNA contained 170 bp in the 3' untranslated region with a typical poly(A) signal43 (AATAAA) that was determined 27 bp upstream of the poly(A) tail. When the full-length nucleotide sequence was analyzed by searching for homologies in the GenBank database, using FASTA, it was observed to have a 72% homology with human and bovine mitochondrial elongation factors,44 EF-Ts.

The presumed initiating ATG was assigned to the first methionine and the predicted protein sequence of 324 amino acid residues was termed the 2A3-2 protein (Figure 6B). The deduced 2A3-2 protein sequence showed a calculated molecular mass of 27 kDa. The sequence was run against the Swiss Prot database, which confirmed the GenBank results. The homologies with the bovine and human EF-Ts were of 85% in the mature protein and of 70% in the overall protein sequence. These important homologies suggest that we have cloned the rat homologous EF-Ts gene. A comparison of our sequence with human and bovine EF-Ts genes is shown in Figure 7. It is noteworthy that the E. coli protein sequence has 29% homology with the bovine sequence. NH2-terminal analysis of the sequence indicates that the rat EF-Ts mature form begins with Ser-41 in the long open reading frame (Figures 6B and 7). The mitochondrial import signal is thus
40 amino acids long and it is 55 amino acids in the bovine form. The mitochondrial import signals are usually not conserved between different species; however, they usually lack acidic amino acids, are enriched in basic and hydroxylated residues, and can form an amphiphilic α-helix or β-sheet. The transit peptide for rat EF-Ts lacks acidic residues and is moderately rich in basic and hydroxylated residues (30%). To summarize, the protein sequence is 324 amino acids (the human protein is 294 amino acids and the bovine one is 338 amino acids), whereas the mature form of the protein is 284 amino acids (283 amino acids for both the bovine and the human homologs). The rat N-terminal part of the protein has 29 amino acids more than the human one.

Discussion

In this study, using differential display, we have isolated for the first time a new gene (2A3-2, 1.2 kb) that is overexpressed in rapidly proliferating, but not synthetic, rat SMC line. Moreover, balloon injury of rat carotids showed, by a virtual northern technique, an upregulation of this new gene in vitro and in vivo conditions. A typical virtual northern blot showing upregulation of 2A3-2 in P200 cells (lane 2) compared with P9 cells (lane 1). The above data, on 2A3-2, confirms those obtained by standard northern blots. Moreover, 2A3-2 is upregulated in a carotid with hyperplasia (lane 5) compared with a healthy carotid (lane 4) and aorta (lane 3). The gene transcript size (1.2 kb) was found to be the same under in vitro and in vivo conditions.

Figure 4. Northern blot experiments showing the upregulation of 2A3-2 under in vitro and in vivo conditions. A, A typical virtual northern blot showing upregulation of 2A3-2 in P200 cells (lane 2) compared with P9 cells (lane 1). The above data, on 2A3-2, confirms those obtained by standard northern blots. Moreover, 2A3-2 is upregulated in a carotid with hyperplasia (lane 5) compared with a healthy carotid (lane 4) and aorta (lane 3). The gene transcript size (1.2 kb) was found to be the same under in vitro and in vivo conditions.

Figure 5. 5′ RACE-PCR amplification. The cDNA library (obtained from the proliferating cells) was used along with a primer coming from the 3′ end of 2A3-2 band. The other primer in the PCR comes from the adapter that is already ligated to the cDNA library. The touchdown PCR technique was used during the 5′ RACE, which allowed us to obtain the 5′ end of the gene. The molecular weight of the 5′ RACE-amplified 2A3-2 gene is 1.2 kb. This confirmed northern results and indicated that we had cloned the full gene. αX174 is given on the left as a molecular weight marker.

Figure 6. Analysis of the different parts of the rat EF-Ts gene (2A3-2). A, The nucleotide sequence (1149 bp) with the poly(A) signal (AATAAA) underlined. The poly(A) tail is designated by (A)n, whereas the initiation and stop codons are shown in boldface (ATG is at +1). It is noteworthy that both human and bovine EF-Ts genes have an intron of 224 bp at position 99 and they have a 5′ untranslated region (5′ UTR) of very short length (18 bp). B, The EF-Ts (2A3-2) predicted protein sequence (324 amino acids) where the methionine (M) and stop (Z) codons are shown in boldface. The mature protein (284 amino acids) starts from the underlined Ser residue at position 41. C, The different reading frames of the gene. Open reading frame 2 contains 972 bp where the ATG on position 8 is the initiation codon. D, Different parts of the gene with the initiation and termination codons. The 5′ UTR is only 7 bp in length and the 3′ UTR is 170 bp.
hyperplasia vessels. The following several lines of evidence support the above statement: (1) Differential display showed an upregulation of 2A3-2 in rapidly proliferating SMCs (V8) but not synthetic (P9) cells. (2) These results, obtained by differential display, were confirmed by northern blots. (3) Multiple-tissue northern showed the presence of this 1.2-kb gene in different rat tissues. (4) SMART technique in tandem with virtual northern blot show 2A3-2 upregulation in balloon-injured carotids compared with controls. (5) 5’ RACE technique allowed us to clone and sequence the full 1.2-kb gene. This new gene shows, on database search, an important homology to human and bovine mitochondrial EF-Ts. Moreover, one should note that EF-Ts is a nuclear encoded gene in mammals. Finally, on a human multiple-proliferating cell line (V8), this gene did not show, at the initial stage of the study, any significant homology to known genes (Zibara et al, unpublished data, 1998). The 2A3-2 cDNA band, after northern blot analysis, was selected for further study, as it was observed to be upregulated in a rapidly proliferating cell line (V8). This gene did not show, at the initial stage of the study, any significant homology to known genes. However, when the full-length nucleotide sequence was obtained, a 72% homology with the bovine and human mitochondrial EF-Ts is observed. Moreover, the protein is 85% homologous to bovine and human mitochondrial EF-Ts. Its importance homology to human and bovine mitochondrial EF-Ts is essential for protein synthesis. Indeed, EF-Ts is a translation factor implicated in the elongation of the ribosome during the elongation cycle of protein biosynthesis. The elongation factor EF-Ts belongs, facilitates the binding of aminoacyl-tRNA to the ribosome. Furthermore, the elongation factor EF-Ts from pig, rat, and human. The protein sequence of bovine EF-Ts is shown in 1-letter code and only the differences are indicated for the 2 other sequences. The mature rat protein starts from the underlined Ser residue at position 41 and constitutes 284 amino acids, whereas both bovine and human homologous EF-Ts proteins are of 283 amino acids. Dots indicates a missing residue; dashes, a similar amino acid.

Vascular SMC proliferation contributes to the pathogenesis of atherosclerosis. In addition, SMC proliferation is a key...
event in neointimal formation after balloon angioplasty and restenosis. The molecular signals that mediate these processes have not yet been identified. This study has identified a new gene, 2A3-2 or EF-Ts, not previously reported to be involved in SMC functions. Further work is needed to delineate the role of this new gene in vascular lesions.

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