

Research Article

6A3-5/Osa2 is an Early Activated Gene Implicated in the Control of Vascular Smooth Muscle Cell Functions

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Vascular smooth muscle cells (VSMC) growth plays a key role in the pathophysiology of vascular diseases. However, the molecular mechanisms controlling gene transcription in VSMC remain poorly understood. We previously identified, by differential display, a new gene (6A3-5) overexpressed in proliferating rat VSMC. In this study, we have cloned the full-length cDNA by screening a rat foetal brain cDNA library and investigated its functions. The 6A3-5 protein shows 4 putative conserved functional motifs: a DNA binding domain called ARID (AT-rich interaction domain), two recently described motifs (Osa Homology Domain), and a nuclear localization signal. The deduced protein sequence was observed to be 85% identical to the recently described human Osa2 gene. Immunolabelling, using an anti-6A3-5/Osa2 monoclonal antibody, showed a nuclear localization of the 6A3-5/Osa2 protein. In addition, PDGF upregulated 6A3-5/Osa2 expression at both the transcript and protein levels in a dose and time-dependent fashion. The pattern of upregulation by PDGF was reminiscent of the early responsive gene *c-fos*. The PDGF-induced upregulation of 6A3-5/Osa2 and proliferation of VSMC were significantly inhibited in a dose and sequence-dependent fashion by an antisense, but not by sense, scrambled or mismatched oligonucleotides directed against 6A3-5/Osa2. In VSMC of aortas derived from hypertensive (LH) rats, 6A3-5/Osa2 is overexpressed as compared to that in normotensive (LL) rats. The 6A3-5/Osa2-gene expression is downregulated by an ACE inhibitor and upregulated by exogenous AngiotensinII in LH rats. In summary, these results indicate that 6A3-5/Osa2 is an early activated gene that belongs to a new family of proteins involved in the control of VSMC growth.

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INTRODUCTION

Vascular smooth muscle cell (VSMC) growth plays a critical role in different pathological conditions such as atherosclerosis [1] and its clinical complications. Indeed, development of these vascular diseases is associated with a loss of vascular contractility counterbalanced by an increase of VSMC migration, proliferation, matrix secretion, and, in some cases, hypertrophy [2]. Different agonists modulate VSMC phenotype and activities in the vessel wall. For example, platelet-derived-growth factor (PDGF), particularly PDGF-BB, stimulates both proliferation and migration [3]. AngiotensinII (AngII), the active biological peptide of the renin-angiotensin system, has potent vasoconstrictor actions

and is directly involved in the development of hypertension. AngII induces a multitude of signalling pathways which, depending on the VSMC phenotype, can lead to contraction, hyperplasia, or hypertrophy [4, 5]. Many transcription factors (such as *c-fos* [6], *Ets-1* [7], *NFκB* [8]) and the subsequent expression of a large number of genes (eg, *alpha-actin*, *Collagen IV*, *MCP-1*, *Endothelin-1*, *PDGF-A*, *TSP-1*, *bFGF*, and *PDGF A-chain* [9]) are stimulated by AngII. However, the molecular mechanisms controlling gene transcription during these processes remain at this stage poorly understood.

A new gene (6A3-5/Osa2), which is overexpressed in proliferating, rat aortic VSMC, was initially identified by differential display [10]. This partially cloned gene of 1.2 kb, not referenced in Genbank, shares sequences homologies with the ARID (AT-rich interaction domain) transcription modulator family. ARID-containing proteins are involved in the

control of transcription during cell growth and embryonic development [11, 12]. However, their precise functions are not fully understood. In the current study, we have cloned the full-length rat 6A3-5/Osa2 cDNA and characterized its deduced protein sequence as a member of the ARID family. Moreover, the knock-down of 6A3-5/Osa2 expression, which is overexpressed in PDGF-dose and time-dependant manner, resulted in a significant reduction of VSMC proliferation. In vivo work showed that 6A3-5/Osa2 is overexpressed in SMC of aortas derived from hypertensive (Lyon hypertensive, LH) but not normotensive (Lyon low-blood pressure, LL) rats. The 6A3-5/Osa2-gene expression is downregulated by an ACE inhibitor and upregulated by exogenous AngII in hypertensive rats.

MATERIALS AND METHODS

Isolation of a full-length 6A3-5 rat cDNA

A 6A3-5 full-length cDNA was cloned by screening a rat foetal brain cDNA library (Origene Technologies, Inc) using primers generated from a previously derived partial sequence (Genbank accession number: AJ005202) [10], combined with in silico analysis of genome databases. BlastA (NCBI) and multiple alignments performed using ClustalW (EBI) were used for assessing sequence homologies.

Cell culture

Primary human and rat VSMC were cultured as previously described [13]. VSMC, at 80% of confluence, were serum starved for 48 h and stimulated by PDGF-BB. Dose-effect (0 to 20 ng/ml) and time-response (0, 2, 4, 8, or 24 h) experiments were performed on human VSMC. Following treatment, VSMC are harvested in Trizol or in lysis buffer (1% of 10 mM aprotinin, 10 mM leupeptine, 10 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride, 25 mM Tris pH 7.6, 150 mM NaCl, and 1% Triton X100).

Immunofluorescence

After fixation and permeabilization (100% methanol at -20°C during 5 min), nonspecific sites were blocked (PBS/3%BSA) for 1 hour at 25°C . The primary antibody (6H3 anti-Osa2 hybridoma supernatant (1 : 5) [14], mouse anti- α -actin monoclonal antibody (1 : 100) or a rabbit anti-NFkB polyclonal antibody (1 : 100), Dako) was incubated for 2 hours at 25°C . After 3 washing steps, VSMC were incubated in the blocking solution with an appropriate secondary antibody-FITC-conjugated (Dako) for 1 hour at 37°C . After 4 washing steps, coverslips were mounted and analysed by fluorescence microscopy.

Northern blot

Total RNA was isolated according to the Trizol procedure. Northern blots were performed as previously described [15]. The abundance of 6A3-5/Osa2 mRNA was normalized with respect to 18 S rRNA and the ratio expressed in arbitrary units (au).

Western blot

Nitrocellulose membrane bearing electrotransferred proteins (30 μg), separated on 7% SDS-polyacrylamide gels, were blocked for 4 hours at 37°C with TBS/ 0.05% Tween20/3% gelatine, and incubated overnight at 4°C with an anti-Osa2 antibody (6H3, 1 : 5 [14]). A swine anti-mouse antibody, conjugated to horseradish peroxidase (Bio-Rad), was then used with a chemiluminescent technique (ECL kitTM, Amersham). Expression level of 6A3-5/Osa2 protein was estimated by Quantity One tool (Bio-Rad) and normalized with Coomassie blue staining.

Gene knock-down by antisense oligonucleotides

The sequences and locations of the generated oligonucleotides targeted against human Osa2 cDNA AF468300 are summarized in Table 1. For transfection experiments, VSMC at 60–70% of confluence were serum-starved for 48 h and then incubated with 25–200 nM ODN at concentration in serum- and antibiotic-free MEM medium in the presence of oligofectamine (Invitrogen). After 4 hours, VSMC were stimulated by PDGF (20 ng/ml) for different periods of time (0, 2, 4, 6, and 24 h) and then harvested in a cell lysis buffer or Trizol. Alternatively, after transfection, VSMC were stimulated by PDGF for 24 hours and used for Bromodeoxyuridine incorporation test (Roche) to estimate cell proliferation.

Animal studies

Protocols for animals' (Lyon hypertensive (LH) and Lyon low-blood pressure (LL) strains) housing and treatment have been previously detailed [16]. Three groups were used: the first group (controls, $n = 8$) was untreated and used as controls. The second group (Ace I, $n = 8$) was treated with an ACE inhibitor, perindopril (3 mg/kg/d), for 4 weeks. The third group (Ace I+ANGII, $n = 8$) was treated with an ACE inhibitor, perindopril (3 mg/kg/d) and perfused subcutaneously with AngII (200 ng/kg/min) for 4 weeks.

Quantification of 6A3-5/Osa2 mRNA by quantitative-PCR

Frozen rat aortas were homogenized at 0°C in 500 μl Trizol and total RNA isolated. Reverse transcription product (Superscript II, *Invitrogen*) was used for quantitative real-time PCR (Q-PCR) on an ABIPrism 7900. Q-PCR assay was carried out using the Assay-on-Demand for 6A3-5, calponin, SM22-alpha, and 18 S mRNA levels were using the comparative Ct method.

Immunohistochemistry

Immunohistochemistry was performed on frozen aorta sections (5 μm) fixed in acetone as previously described [15] with an anti-Osa2 hybridoma supernatant (6H3) [14] or an anti- α -actin (Dako) monoclonal antibody. Primary antibody binding was detected using a secondary antibody

TABLE 1: Homologies of rat 6A3-5 sequences with ARID proteins. Homologues are divided into two subgroups. The first subgroup, which would define the subfamily called Osa, has members bearing an ARID motif and two OHD domains. The second subgroup indicates different ARID proteins.

	Species	Yeast	Drosophila	Mouse	Human
Subgroup 1	Names	Swi1	Osa, <i>eyelid</i>	Osa1	Osa1, P270, B120, BAF250
	Accession no	M84390	AF053091	AF268912	AF521670
	Chromosome no	—	3	4	1p35-p36
	cDNA Length	3027 bp	10601 bp	7041 bp	6418 bp
	Protein Length	825 aa	2715 aa	1902 aa	1999 aa
	Functions	– Member of yeast SWI/SNF	– Member of Brahma complex – Antagonize wingless pathways	– Interaction with Brahma chromatin remodelling complex	– Member of human swi/snf – Co-factor of transcriptional activation by the steroid hormone receptors
	Name	nd	nd	nd	Osa2, held/Osa1, KIAA1235
	Accession no	—	—	—	AF521671 and AF468300
	Chromosome no	—	—	—	6q25.1–q25.3
	cDNA Length	—	—	—	5482 pb
Protein Length	—	—	—	1740 aa	
Functions	—	—	—	– Member of human swi/snf – Promotes transcriptional activation by the steroid hormone receptors	
Subgroup 2	Name	nd	Dead Ringer (Dri)	Bright	DRIL-1
	Accession no	—	U62542	U60335	U88047
	Chromosome no	—	—	10	19p13.3
	cDNA Length	—	3696 bp	4842 bp	2725 pb
	Protein Length	—	901 aa	601 aa	593 aa
	Functions	—	– Embryo patterning – Target sequence: AGATT/ATAA	– B-Cell activator – Target sequence: AGATTAA	– Binds the pRb controlled transcription – Target sequence: A/GATT/ATAA
	Name	nd	nd	Mrf2	Mrf2
	Accession no	—	—	AF280065	M733837 (partial sequence)
	Chromosome no	—	—	10	10
	cDNA Length	—	—	3647 bp	—
Protein Length	—	—	1188 aa	—	
Functions	—	—	– Accumulation of lipids in postnatal life – Target sequence: AATA(C/T)	—	
Name	nd	nd	Jumonji	Jumonji	
Accession no	—	—	BC05244	U57592	
Chromosome no	—	—	—	6q24.p23	
cDNA Length	—	—	4939 bp	—	
Protein Length	—	—	1324 aa	1266 aa	
Functions	—	—	– Neural embryogenesis	Highly expressed by neuron cells during development	

TABLE 2: List of antisens oligonucleotides directed against 6A3-5. Sequence and position (on human homologous held/Osa2) of different ODN directed against 6A3-5. Only ODNAS3 showed significant effects on 6A3-5 expression. Scrambled ODN3 (ODN Scr3) and mismatched (ODN Mis3) as used to test sequence specificity of ODNAS3.

Name	Sequence 5'–3'	Position/AF468300
ODNAs1	agcttgctgaacttactggct	3870–3890
ODNAs2	cagcttgctgaacttactggctt	3869–3889
ODNAs3	tgggatctgccatg	57–71
ODN Scr3	agctcggttcacggt	—
ODN Mis3	agggagctaccc ctg	57–71
ODNAs4	tcacatctgagaatgg	2245–2260

conjugated to horseradish peroxidase followed by 3-amino-9-ethylcarbazole (Dako). The specific location of the α -actin in the media of aorta was used to define the medial boundaries. The media thickness was then measured at a magnification of X40 in slides counterstained with Haematoxylin (Dako).

RESULTS

Cloning of full-length rat 6A3-5 cDNA

The cloned gene has a 6569 bp cDNA sequence (GenBank accession number: AJ440711) and a deduced amino acid sequence corresponding to a 5276 bp open-reading frame (Figure 1(a)). The cDNA contained 1268 bp in the 3'-untranslated region; the 5'-untranslated sequence is not totally cloned. The putative 1758 amino acid 6A3-5 protein has an expected molecular weight of 180 kDa and bears four conserved motifs (Figure 1(b)). The first motif is a DNA binding domain, called AT-rich interaction domain or ARID, located in the N-terminal half (aa 568 to 672). Two other motifs comprising evolutionary conserved domains known as OHD (Osa Homology Domain)-1 (aa 1114 to 1200) and OHD2 (aa 1437 to 1758) are present within the C-terminal half of the protein. These three motifs are the signature of a novel family of transcription modulators called Osa family and indicate that 6A3-5 is the rat Osa2 homologue. Finally, a fourth motif represented by a nuclear localization signal is also present in the C-terminal of 6A3-5/Osa2 sequence suggesting a nuclear localization of this protein that was subsequently confirmed.

Multiple sequence alignment and homologies to rat 6A3-5/Osa2

Protein similarity searches revealed two subgroups with significant homologies to rat 6A3-5/Osa2 protein. The 1st subgroup comprises proteins bearing ARID, OHD1, and OHD2 functional domains. This group shows a remarkably high degree of conservation of amino acid sequences, and includes the recently cloned human Osa2 [14]. This protein appears to be the human orthologue of rat 6A3-5/Osa2, mouse, and human Osa1 [17], *Drosophila* Osa/eyelid [18]

and yeast SWI1 protein [19] (Figure 2). The 2nd subgroup shows homologies that are limited to the ARID domain and include *Drosophila* dead-ringer protein [20], its homologues in mouse (bright) [21] and human (DRIL-1), mouse Mrf2 [22], and the murine and human jumonji proteins (Table 2).

Cellular localization

VSMC characterized with anti- α -actin antibody showed its nucleus to be equally labelled with an anti 6A3-5/Osa2 [14] or an anti-NFKB antibody. Negative controls showed no labelling (Figure 3).

6A3-5 expression in different phenotype of vascular SMC

Transcription levels of 6A3-5/Osa2 and α -actin markers were measured after dedifferentiation of ex vivo SMCs from a contractile (passage 0, P0) to an in vitro synthetic phenotype (passage 9, P9). Northern-blots showed that 6A3-5 is up-regulated by 3-fold ($n = 3$) in the synthetic phenotype in comparison to the contractile quiescent phenotype. In contrast, α -actin expression is present in the contractile SMCs phenotype and lost on differentiation to a synthetic phenotype (Figure 4(a) and data not shown) [23]. The 6A3-5/Osa2 gene was significantly upregulated in a smooth muscle cell line (V8) that was observed to be highly proliferating [24] compared with secretory/ synthetic cells (results not shown).

Time course and dose effect of PDGF on 6A3-5/Osa2 in VSMC

Human and rat (data not shown) VSMC were serum starved, inducing a down-regulation of 6A3-5/Osa2 mRNA expression levels, and then treated with 20 ng/ml of PDGF-BB for 0, 2, 4, 8, and 24 hours. Northern blot analysis showed that the levels of 6A3-5/Osa2 mRNA reached a peak at 2 hours and remained above the control level for at least 24 hours after PDGF treatment (Figure 5(a)). In addition, a PDGF dose-dependant effect was also observed with a maximal increase achieved at 20 ng/ml (Figure 5(b)). Similar results were observed at 4 hours, by Western blot, for 6A3-5/Osa2-protein expression (Figures 5(c), 5(d)).

Antisense ODN inhibition of 6A3-5/Osa2 expression and VSMC proliferation

A series of 20-base phosphorothioate antisense ODN (Table 1, ODN_{AS1-4}) was screened for its ability to selectively inhibit 6A3-5/Osa2 protein expression in human VSMC. After transfection, VSMC were stimulated by PDGF-BB for 4 hours. The ODN_{AS3}, which hybridizes to the 6A3-5/Osa2 ATG translation initiation site, showed a significant inhibition of 6A3-5/Osa2 mRNA and protein expression in comparison to its sense, scrambled, and mismatched controls (Figures 6(a), 6(b), 6(c)). Moreover, treatment of human VSMC with increasing concentrations of ODN_{AS3}

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1  agccccggca  ccctcgacc  gaccatgggc  agatcccagg  gcagtccgat  ggaccaatg
61  gtgatgaaga  gacctcagtt  gtatgggatg  ggcaactcacc  cccattcgca  gccgcagcag
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241  cctcagtatg  gcagcaaaag  tgtgagtgg  tactgccagc  agggccaaca  gccatattac
301  agccagcagc  cgcagccccc  gcaacctcca  ccccaggcgc  agtatctgcc  gtcccagtc
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841  catgctggaa  tcagtgcgct  tcagcagagt  aactcaagtg  ggacttacgg  tccacagatg
901  agccagtatg  catttccaa  taactactcc  agaccccag  cgtatagtg  ggtgccaggt
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1021  caagggcaaa  gccagccatg  tgggtctgtg  cccctgggac  gaatgccatc  agctgggatg
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1141  cagcagggag  ggccaggaat  gggccgggca  ccaggcccac  caatgcccac  tgtgaaccgc
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1261  caaggcagct  ttccctggca  gcaccagagt  ggactcgtgg  cctccagctc  tccctacagc
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1381  cccacctagg  tgaacagctc  tacagcatct  atgggtctta  cagatagtat  gttcccaggt
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1501  gagagcaagt  caaaggatag  ctacagctct  cagggtatct  ctccagctcc  aactcccagg
1561  aacctgccag  tcccttcccc  aatgtcccc  agctctgcca  gcatctctc  ctctccagga
1621  gatgagatg  acagcattag  cagcccaggc  tggcccaaga  ctccatcaag  ccccaagtcc
1681  agctcctct  ccaccactgg  ggaaagatc  acaaaagtgt  acgagctggg  gactgcgccg
1741  gagaggaagc  ttgggtcga  ccgctacctc  acattcatgg  aggagagggg  gtcccctgtg
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1861  aaagagatcg  gaggttggc  gcaggttcat  acaaaacaaga  agtggcgca  gctggcaacc
1921  aaccgcaagc  ttggcacttc  gagcagcga  gccagctccc  cgaagaagca  atatttcag
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2041  agcacggggg  atgcgaagaa  gcagcccagg  ctccagccgc  catctctctg  caactcggga
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2161  ggcgaccgca  agccaccaac  cccagcctcc  acccctcagc  gacaggggac  ccccatgcaa
2221  agcgggaaga  gcagtcagct  cagtgtgcac  gaccctctc  cagacgtgag  tgatcagcg
2281  tacccaaac  ggaactccac  gactccaaac  gccccatacc  agcagggcat  gggcatgcca
2341  gacatgttgg  gcaggatgcc  ctatgcgccc  aacaaggacc  ctctcagtg  aacgagaaaa
2401  gtgcctggaa  gcagcagacc  ctttatgaca  caaggacaga  tgcccacaag  cagtatcgag
2461  gacatgtaca  accagagtc  ctccagtgcc  atgtccaatc  tgggcatggg  acagcggcag
2521  caatttcct  atggaaccag  ttacagcga  aggcagcagg  ctacgggca  gcagtaccca
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3121  tccatgcaac  ccatcagcgc  cccacctcag  tcatctacc  agacggccc  gtcactgcca
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3241  tctccaagca  agtccccct  cctgctgcc  atgaagatg  agaaggtcat  gccacggtc
3301  cccacatccc  aggtcaccgg  accacccca  cagccaccgc  caatcagaag  ggagattacc
3361  ttctctctg  gctcagtaga  agcatcgag  ccagtcccga  aacaaaggcc  gaagattacc
3421  tcaaaagata  ttgttactcc  cgaggcgtgg  cgtgtgatga  tgtcccttaa  atccggtctg
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4141  gattctgaag  atgcagcaga  gaaaagcacc  atagcccaca  tcgatgatgt  cctgtctgcc
4201  cgaccagggg  cctgcccgga  agacagcaac  cccggacccc  aaaccgagag  cgcaagttt
4261  cccttggaa  tccagcaggc  caaaagccac  cggaaatca  ggctcctgga  ggacagacc

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(a)

FIGURE 1: Full-length ORF sequence of rat 6A3-5, 6569 pb. (a) The full-length rat 6A3-5 cDNA has a first methionine that corresponds to an ATG codon (position 25 underlined) and a stop codon (TGA, position 5301) followed by polyadenylation signal (position 6299). (b) ORF translation sequence with 1758 aa. Conserved motifs are indicated: ARID motif (568–672) is underlined, OHD motifs (OHD1: 1114–1200; OHD2: 1437–1758) are boxed, and NLS (nuclear localization signal) (1352–1369) in bold.

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4321 agggagccgag acgagactcc cctgtgcacc attgcgcact ggcaggactc cctggccaaa
4381 cgctgcatct gtgtgtccaa catcgtgcgg agcttgtctt tcgtgcctgg caacgacgca
4441 gagatgtcca aacatccggg cttggtgccc atcctagtaa agtcgattct gctgcatcat
4501 gagcatccgg agagaaaacg ggcgccacag acctatgaga aggaggagga tgaggacaag
4561 ggggtggcct gcagcaaaaga tgagtgggtg tgggactgcc tcgaggtctt gcgggacaac
4621 acactgggtca cattggctaa cattieccggg cagctagact tgtctgctta cacagagagc
4681 atctgcttgc cgatcctgga cggcttgctg cactggatgg tgtgcccgtc tgacagggca
4741 caggaccctt tcccactgt ggggcccacac tcagttctat cggccacaag acttgtgctg
4801 gagacactgt gtaaaactcag tatccaggac aacaatgtgg acctgatctt ggccacacct
4861 ccatttagtc gtcaggagaa attttatgct acattagtta ggtacgttgg ggtacgcaaa
4921 aaccagctct gccgagaaat gtccatggcg cttttatcga accttgccca gggggataca
4981 ctggcagcga gggcaatagc tgtgcagaaa ggaagcattg ggaacttgat aggtctctg
5041 gaggacgggg tcacgatggc gcagtatcag cagagccagc ataccctcat gcatatgcag
5101 cccccgcctc tggaaacccc cagtgtggac atgatgtgca gggcgcccaa agctttgctg
5161 gctatggcca gagtggatca gaaccgctcg gaggttcctt tgcacgaggg tcggttctg
5221 gatatctcga tatccgctgt cctgaactct ctggttgcgt ctgtcatctg tgatgtactg
5281 tttcagattg ggcagttatg acaccctgga gggcacacat gtgtgagggg acattagagg
5341 gtcacatatg actggctggt tctgttctc gtttatccaa tgtaggaaga aggaaaagaa
5401 aatcttttgc tctctgccc cactcaactat ttaccaattg ggaataaag aatcatttaa
5461 tttgaaacgt tataaattaa tatttgcgtg ctgtgtgtat aagtacatcc gttgggggat
5521 tctcttttct ttctcttttt ttaaccacaa gttgcccgtt agtgcattca caggtcacat
5581 gtttttttgt ttttttcata attttttcca tgttgtatta cagtttttagg gaagtgaatt
5641 cactttataa agtaaaaagg tttggcaaaa aatgtctgata ggaaaatttc accacactga
5701 gtcaaaaagg tgaaggaaa aattgatcct taaattgatt tcctatgaa tttattcttc
5761 gcagaatgaa aaaagcgaag gtgcatccca ttgccaaaag ctctgtgcaa tagaaacttc
5821 tagagatgta ggtgtagggg ctcgaggtat ggcagtcagc agtctggccc agtgatgctg
5881 ttctctccac aggaagcggg ttgcattagg cctcagacaa aaaaaccgac tctcagttag
5941 ggggtgaaat ccactcctaa ccgccaacag caggattgct tctcaccac gaccgcatg
6001 tctgtctgca ctcagcctcc acctcacaga tcttctgcat tcttctcact attttttaa
6061 tatttttttt ttactgctta tgggctgtga tgtatataga agttgtacat taaacatacc
6121 ctcataattt ttctttttct tttttttttt ttagtacaaa gtttttagtt tctttttcat
6181 gatgtgtaaa ctacgaagtg atggttagatt taataaattt tttattttta ttttataat
6241 tttttcatta ggaccatata tccaaaaaac aagaaaaaga acaaaaaaat tacaaaaaat
6301 taacaaaaca aaaaaagggg gtaattgtaca agtttctgta tgtataaagt catgctctgt
6361 tgggagggca gctggtccca atttgcttca tgaatcaagg tgtggaaatg gttgcatagc
6421 gattgattta gaaaatgaat accagttact acaaaaaaaa agaaaaaaga aaaaccaact
6481 aatgaagaa acacaacttc aaagattttt ctgtgacaag aatccgcatt tgtatttcaa
6541 gataatgtag ttttaaaaaa aaaaaaaaaa

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(a)

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1 MGRSQGSPMD PMVMKRPQLY GMGTHPHSQP QSSSPYPGGA YGPPGAQRYP LGMQGRAPGA
61 LGGLQYPQQQ MPPQYGGQGV SGYCCQQGQP YYSQQPQPPH LPPQAQYLPQ SQSQRYQPQQ
121 DMSQEGYGR SQPPLAPGPK NHEDLNLIQQ ERPSLLPDLG GSIDDLPTGT EATLSSAVSA
181 SGSTSSQGDQ SNPAQSPFSP HASPHLSSIP GGPSPSPVGS PVGNSQSRSG PISPASIPGF
241 MAGTQRNPQM AQYGPQQTGP SMSPHSPGG QMHAGISRFO QSNSSGTYGP QMSQYGPQGN
301 YRPPAPYSGV PSASYSGPGP GMGISANNQM HGQGPSQPCG AVPLGRMPGA GMQNRPPFPGN
361 MRSMPSSSPG MSQGGGPGMG RAPGPPMPV NRKAQEAANA VMQAAANSAQ SRQGSFPGMH
421 QSLVAVSSP YSQPMNNNSN LMGTQAQPYIS IPTMVNSST ASMGLTDMMS PVSVKLSVPL
481 KADGKEEVP QPESKSKDSY SSQGISQPPT PGNLFPVSPM SPSSASISSF HGDSDSISISS
541 PGWPKTPSSP KSSSSSTTGE KITKVEYELGT APERKLWVDR YLTFMEERGS PVSSLPVAVGK
601 KPLDLFRLYV CVKEIGGLAQ VHTNKKWREL ATNPNVGTSS SAASSPKKY IQYLFAFECK
661 IERGEPPPE VFGTGDAAKQ PKLQPPSPAN SGLQGPQTP QSTGSSMAE VPGDKPPTP
721 ASTPHGQGT MQRSSSTVS VHDPPSDVD SAYPKRST PNPYQGMG MPDMLGRMP
781 APNKDPFSGT RKPVGSSEPF MTQGGMPNS MDMYNQSPS GAMSNLGMG RQFPYGTYSY
841 DRRHEAYGQ YPGQPPTGQ PYPGGHQPGL YPQQPNYKRH MDGMYGPPAK RHEGDMYMQ
901 YGSQQEYMYN QYGGYSYSGP RRPYQGYPY PYNRERMGP GQMOTDGIIP HMMGGPMQSS
961 SNEGPPQNMW ATRNDMPYYP QNRQGGGGA QAPPYPMNR TDHMMVPDQR INHESQWPSH
1021 VSQRQPYMSS SASMQPITRP PQSSYQTPPS LPNHISRAPS PASFPRLSLES RMSPSKSPFL
1081 PAMKMQKVMV TVPTSQVTGP PPQPPPIRRE ITFPPGSVEA SQVPVKQRRK ITSKDITVPE
1141 AWRVMMSLKS GLLAESTWAL DTINILLYDD STVATFNLSQ LSGFLELLVE YFRKCLIDIF
1201 GILMEYQVGD PSQRALDHRG GKKDDSSQSE DSGKKEEDA ECLEEEEEEE EEEEEEEQV
1261 SKKTESEGKS SSALAAPDIT ADPKETPRQA SKFDKLPKI VKKNNLFVVD RSDRLGRVQE
1321 FNSGLLHWQL GGGDTTETHIL THFESKMEIP FRRRFPAPLS STGKKKELAG KGDSEEQPEK
1381 SIIATIDVSL SARPGALPED SNPGPQTESG KFPFGIQQAK SHRNIRLLED EPRSRDETPL
1441 CTIAHWQDSL AKRCICVSNV VRSLFVPGN DAEMSKHPL VPILGKSILL HHEHPERKRA
1501 PQTYEKEEDE DKGVACSKDE WWWDCEVLR DNTLVTLANI SGQLDLSAYT ESICLPILDG
1561 LLHMVCPESA EAQDPFPTVG PNSVLSQRL VLETLCLSI QDNNVDLILA TPFPSRQPKF
1621 YATLVRYVGD RKNPVCREMS MALLSNLAQG DTLAARAIAV QKGSIGNLIG FLEDGVTMAQ
1681 VQSQHTLMH MQPPPLEPPS VDMCRAAKA LLAMARVDQN RSEFLHEGR LLDISISAVL
1741 NSLVASVICD VLFQIGQL

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(b)

FIGURE 1: Continued.

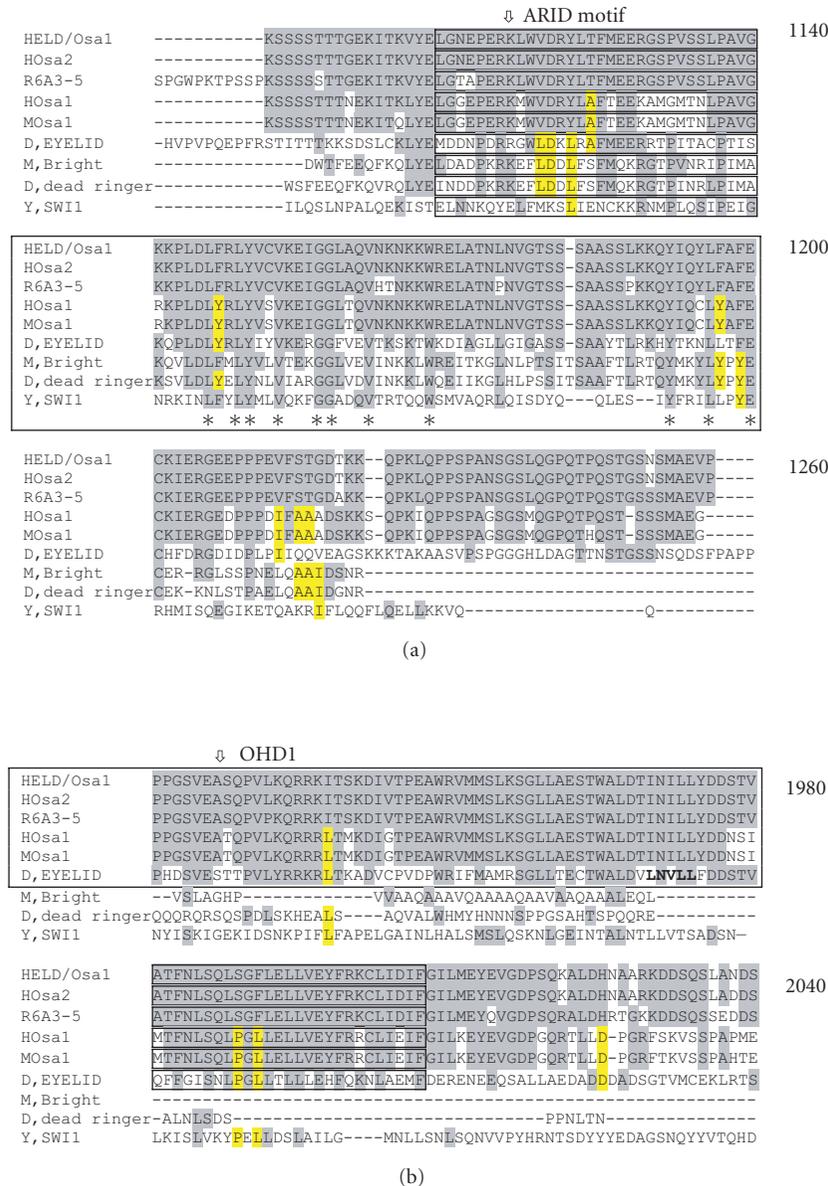


FIGURE 2: Alignments of conserved domains of ARID proteins by CLUSTALW program. (a) ARID motif alignment among different species is shown. (b) OHD-1 (Osa homology domain-1) motif alignment. (c) OHD-1 (Osa homology domain-1) motif alignment. Conserved aa residues are shown by underlining. Identical residues are indicated in grey. H: human, r: rat, M: Mouse, d: Drosophila, Y: yeast (HELD/Osa1 or hOsa2: human homolog of 6A3-5/Osa2).

(25, 100, 200 nM) resulted in a dose-dependent reduction in 6A3-5/Osa2 protein level (Figure 6(d)) but had no effect on P53 expression. Indeed, 6A3-5/Osa2 expression is reduced by 60–70% in presence of 200 nM of antisense ODN_{AS3}.

To investigate whether reduction of 6A3-5/Osa2 expression affected PDGF-induced proliferation, serum starved human VSMC were exposed to ODN_{AS3} and then stimulated by PDGF-BB for 24 hours. ODN_{AS3} reduced by 50–60% PDGF-induced proliferation in human VSMC (Figure 7(a)) while sense, scrambled, or mismatched oligonucleotides derived from ODN_{AS3} had no effect. Moreover, increasing

the concentration of ODN_{AS3} significantly reduced PDGF-induced proliferation of VSMC in a dose-dependent manner (Figure 7(b)).

Expression of 6A3-5/Osa2 and vascular Phenotype in LH versus LL rats

Quantitative PCR performed on aorta excised from hypertensive (LH) rats exhibited significantly increased 6A3-5/Osa2 gene expression levels compared to those present in normotensive (LL) rats (Figure 8(a)). A significant decrease in VSMC contractile markers, calponin and

		↓ OHD2	
HELD/Osa1	KFPFGIQQAQS-----	HRNIKLEDEPRSRD	ETPLCTIAHWQDSL
Hosa2	KFPFGIQQAQS-----	HRNIKLEDEPRSRD	ETPLCTIAHWQDSL
R6A3-5	KFPFGIQQAQS-----	HRNIRLEDEPRSRD	ETPLCTIAHWQDSL
Hosa1	KFPFGISPAQS-----	HRNIKILEDEPHSKD	ETPLCTLLDWQDSL
Mosa1	KFPFGISPAQS-----	HRNIKILEDEPHSKD	ETPLCTLLDWQDPLA
D, EYELID	RLTNGVAPCSSTPAIFDPRTTAKDEARVLQRRRDSSEFEDECYTRD		EASLHLVSESDSLA
M, Bright	-----	-----	EKKMALVADEQQRIM
D, dead ringer	DYAHG-----	-----	EHNNTGNSSSMHDDSEPOQMN
Y, SWI1	ILNFKKDLVIVLS-----	NISHLLEIASSIDCLLI	LILVISFGQPKL
HELD/Osa1	KRCICVSNIVRSLSFVPGNDAEMSKHPGL	VLILGKLILLHHEHPERKRAPQTYEKEEDE	
Hosa2	KRCICVSNIVRSLSFVPGNDAEMSKHPGL	VLILGKLILLHHEHPERKRAPQTYEKEEDE	
R6A3-5	KRCICVSNIVRSLSFVPGNDAEMSKHPGL	VPILGKSI LLHHEHPERKRAPQTYEKEEDE	
Hosa1	KRCVCVSNIRSLSFVPGNDFEMSKHPGL	LLILGKLILLHKKHPERKQAPLTYEKEEQ	
Mosa1	KRCVCVSNIRSLSFVPGNDFEMSKHPGL	LLILGKLILLHKKHPERKQAPLTYEKEEQ	
D, EYELID	RRCIALSNIFRNLTFVPGNETVLAKSTRF	LAVLGRLLLNHEHLRTPKTRNYDREEDT	
M, Bright	QRAVQQS-FLAMTAQLPMN-----	IR----	INSQASESRQDSAVSLTSANG
D, dead ringer	HHHHQTHLHDKDDSAIENSPTTSTTTG-----	GSVGRHSSPVST	KKGGAKPOQSGK
Y, SWI1	NPMASSSFGSESLTFNEFQLQWGYQTFGVD	LAKLFS	LEKPNLNYFKSI LNKNTGNN
HELD/Osa1	DKGVACS----KDEWWWDCLEVLRDNTLVTLANISGQLDLSAYTESICLPI	LDGLLHWMV	
Hosa2	DKGVACS----KDEWWWDCLEVLRDNTLVTLANISGQLDLSAYTESICLPI	LDGLLHWMV	
R6A3-5	DKGVACS----KDEWWWDCLEVLRDNTLVTLANISGQLDLSAYTESICLPI	LDGLLHWMV	
Hosa1	DQGVSCN----KVEWWWDCLEMLRENTLVTLANISGQLDLSAYTESICLPI	LDGLLHWMV	
Mosa1	DQGVSCD----KVEWWWDCLEMLRENTLVTLANISGQLDLSAYTESICLPI	LDGLLHWMV	
D, EYELID	DFSDSCSSLQGEREWWWDYLITIRENMLVAMANTAGHLELSRDELLARELIDGLLHWMV		
M, Bright	SNSISMS-----	VEMNGIVYTGVLFAQPP-----	PPT
D, dead ringer	DLPTEDK-----	DASSGKLNPLETSLLSG-----	MQFQV
Y, SWI1	LYDRNSN-----	NNHKDKLLRRLNLYDNNKNNNNRHNLLNDVVVFLFSAIP	QOVL
HELD/Osa1	CPSAEAQDPFPTVGPNSVLSPQRLVLETLCKLSIQDNNVDLILATPPFSRQEKFYATLVR		
Hosa2	CPSAEAQDPFPTVGPNSVLSPQRLVLETLCKLSIQDNNVDLILATPPFSRQEKFYATLVR		
R6A3-5	CPSAEAQDPFPTVGPNSVLSPQRLVLETLCKLSIQDNNVDLILATPPFSRQEKFYATLVR		
Hosa1	CPSAEAQDPFSTLGPNAVLSPQRLVLETLCKLSIQDNDVDLILATPPFSHLEKLYSTMVR		
Mosa1	CPSAEAQDPFSTLGPNAVLSPQRLVLETLCKLSIQDNNVDLILATPPFSRLEKLYSTMVR		
D, EYELID	CPSAHGQDPFPCGPNVLSPQRLALEALCKLCVTDANVDLVIATPPFSRLEKLCVAVLIR		
M, Bright	APSA PGKGGVSSIGTN-----	TTTG--SR	
D, dead ringer	ARNTGDNGEQQLIVNLELNGVKYS-----	G-----	VLVANVLSQ
Y, SWI1	SQSADPSLLIDQFSPVISQSLTSLVIVQKILPLSNEVFEISENNSDSNSNN		
HELD/Osa1	YVGDRKNPVCREMSMALLSNLAQGDALAARAIAVQKGSIGNLISFLEDGVTMAQYQSQSH		
Hosa2	YVGDRKNPVCREMSMALLSNLAQGDALAARAIAVQKGSIGNLISFLEDGVTMAQYQSQSH		
R6A3-5	YVGDRKNPVCREMSMALLSNLAQGDALAARAIAVQKGSIGNLIGFLEDGVTMAQYQSQSH		
Hosa1	FLSDRENVPCREMAVLLANLAQGDSLAARAIAVQKGSIGNLLGFLEDSLAATQFOQSQA		
Mosa1	FLSDRENVPCREMAVLLANLAQGDSLAARAIAVQKGSIGNLLGFLEDSLAATQFOQSQA		
D, EYELID	HLCRNEQVLRFSVNLHLYLAADSAMARTVALQSPCISYLVAFIEQAEQTLGVANQH		
M, Bright	-----TG-----	ASGSIVSGGQVLPVSTPTMSSTNS	SLP-----
D, dead ringer	-----	SETRTSSPCHAEAPTVEEEKDEE	EEEEEPKAAEEES
Y, SWI1	--GNKDSSFNFKNLPFVWLSSEENIGSGLLKLSEIILNINNSTSKNTLLQQNYSKVL		
HELD/Osa1	NLMHMQ-PPPLEPPSVDMMCRAAKALLAMARVDENRSEFLLHEGRLLDISISAVLNSLVA		
Hosa2	NLMHMQ-PPPLEPPSVDMMCRAAKALLAMARVDENRSEFLLHEGRLLDISISAVLNSLVA		
R6A3-5	TLMHMQ-PPPLEPPSVDMMCRAAKALLAMARVDQNRSEFLLHEGRLLDISISAVLNSLVA		
Hosa1	SLLHMQ-NPPPEPTSVDMRRRAARALLALAKVDENHSEFTLYESRLLDISVSPLMNSLVS		
Mosa1	SLLHMQ-NPPPEPTSVDMRRRAPRALLALAKVDENHSEFTLYESRLLDISVSPLMNSLVS		
D, EYELID	GINYLRENPDMSGTSLDMLRRAAGTLLHLAKHPDNRSLFMQQEQRLGLVMSHILDQQVA		
M, Bright	-----	-----	-----
D, dead ringer	HRSPVK---QENEDADQDMEGSEVLLNGGASAVGG--AGAGVG--VGVG	VPLKDAVVS	
Y, SWI1	LPSINISCVQITKCLVEKSI CFENCLNNDPEILKKTIASIPNLFPTDLEIFQLETFNPSVDI		
HELD/Osa1	SVICDVLFIQIGQL-----		
Hosa2	SVICDVLFIQIGQL-----		
R6A3-5	SVICDVLFIQIGQL-----		
Hosa1	QVICDVLFIIGQS-----		
Mosa1	QVICDVLFIIGQS-----		
D, EYELID	LIIISRVLYQVSRGTGPIHSVFRLLQQRQQQLRPGPAGQAASAGGSATVKAETASTET		
M, Bright	-----	-----	-----
D, dead ringer	-----	-----	-----
Y, SWI1	QIINQYQILYNLKNIDILTNL-----		

(c)

FIGURE 2: Continued.

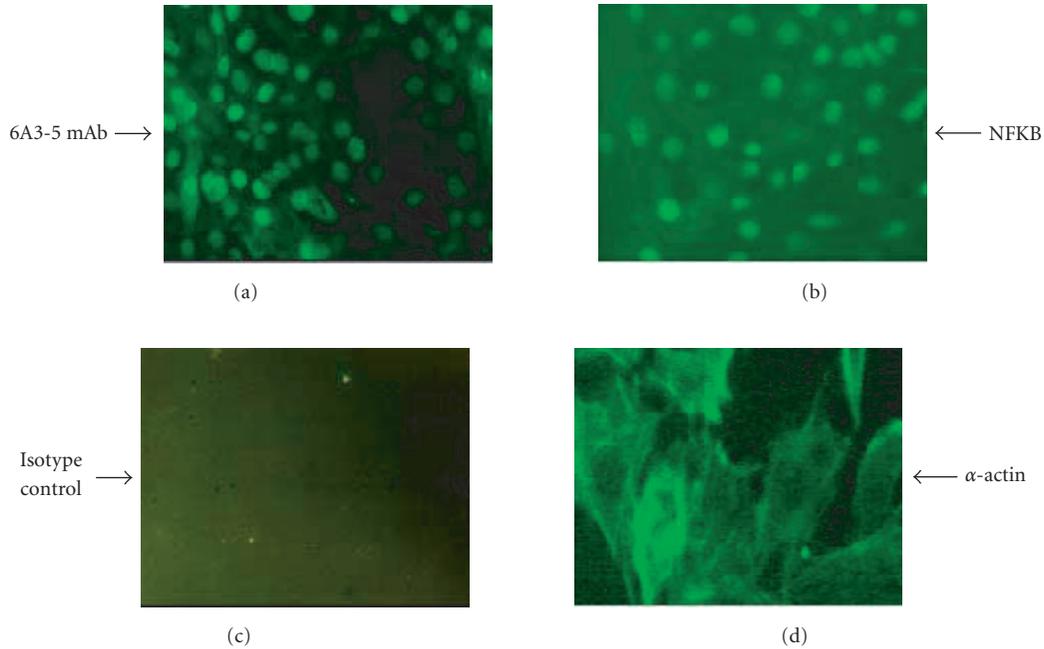


FIGURE 3: 6A3-5/Osa2 cellular localization on VSMC. Actin was used as a cytoplasmic control of smooth muscle cells marker, while NFKB served as a nuclear control. Cell nucleus was labelled with an anti-6A3-5/Osa2 or an anti-NFKB antibody in comparison to isotype control (mouse IgG).

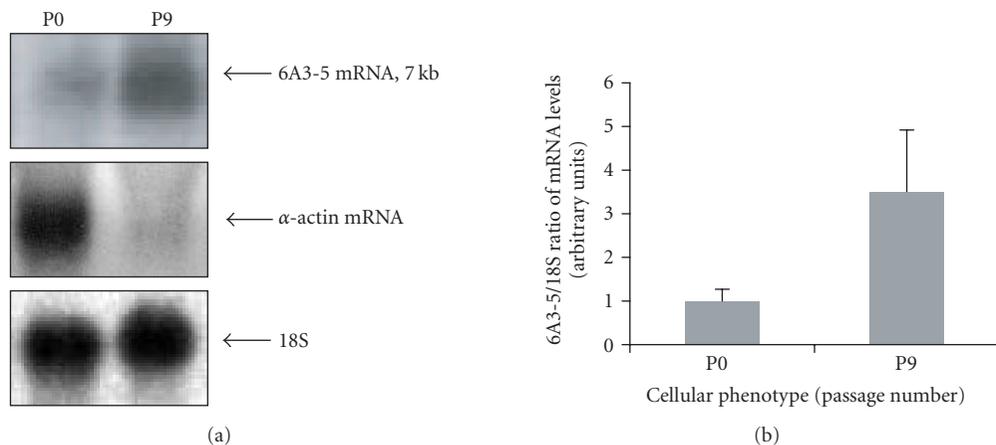


FIGURE 4: 6A3-5 and α -actin SMC expression in different vascular SMC. (a) Levels of 6A3-5 gene transcription were compared, by northern blot, between the contractile (passage 0, P0) and the secretory/synthetic phenotype (9th passage, P9). Phenotypes were characterized by the α -actin SMC marker. The 18 S served as a control for loading and quantification. (b) Quantification of 6A3-5 signals, done on 3 independent northern blots, reported to the 18 S levels. Results show 6A3-5 mRNA levels to be increased by 3 folds in synthetic cells compared to contractile cells.

SM22-alpha, was observed in LH but not LL rats (Figures 8(b), 8(c)).

Immunolabelling indicated the presence of 6A3-5/Osa2 in VSMC of LH and LL aortas (Figures 9(a), 9(b)), but no labelling was observed in negative controls (Figure 9(e)). Interestingly, the 6A3-5/Osa2 antibody shows similar labelling

to those observed with proto-oncogene c-fos (Figures 9(c), 9(d)). Such an increased level of 6A3-5/Osa2 was associated with a state of hypertension. Indeed, work by Aguilar et al [16] has shown that LH rats have a systolic blood pressure (SBP) of 166 ± 3.59 compared to 131 ± 2.78 mmHg for LL.

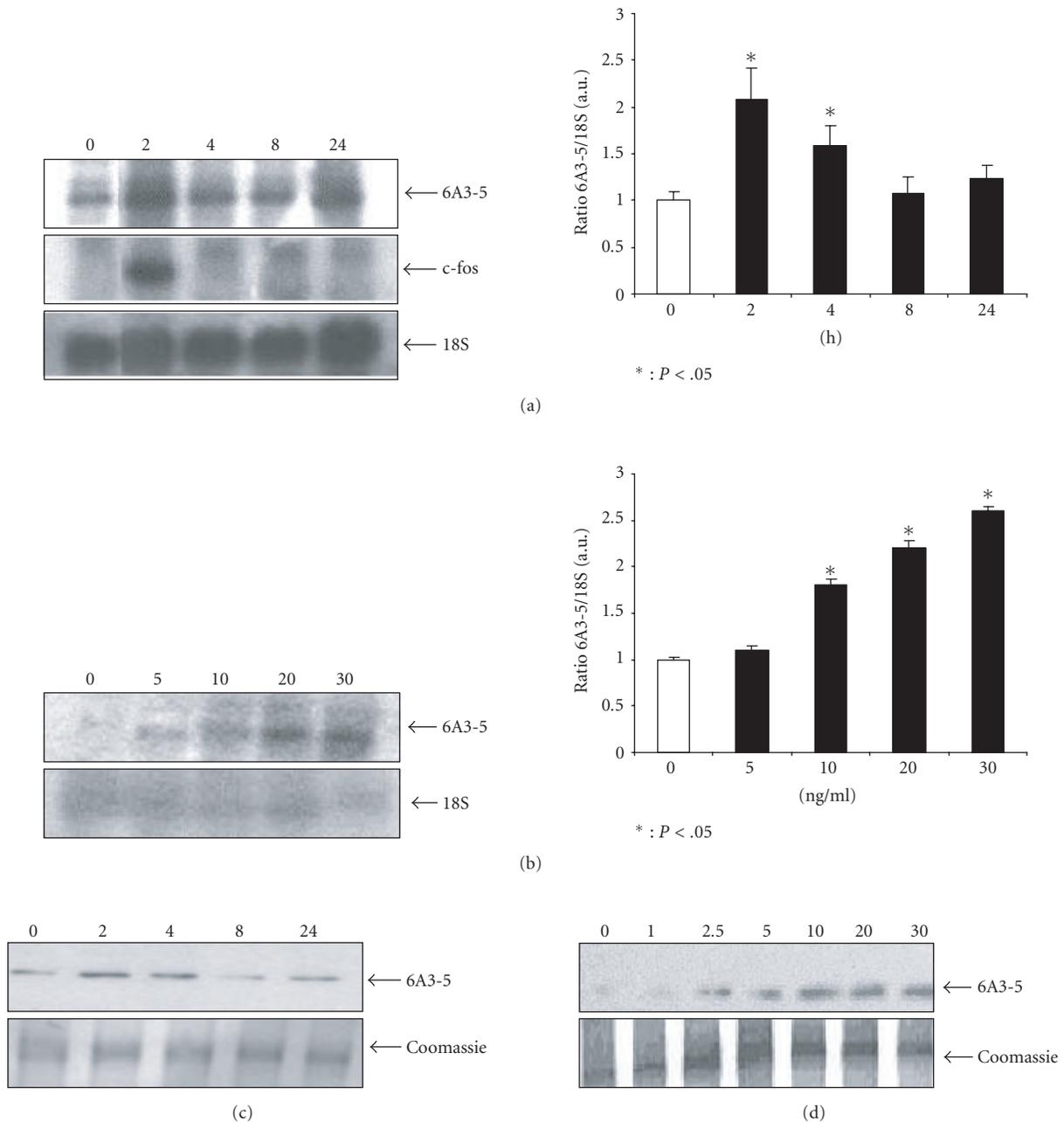


FIGURE 5: Time course and dose effect of PDGF on 6A3-5/Osa2 expression. (a) Time course of 6A3-5/Osa2 mRNA level analysed by Northern-blot, following treatment of human proliferating VSMC (9th passage) with 20 ng/ml PDGF. VSMC were serum starved for 48 hours before analysis. (b) Dose effect of 6A3-5/Osa2 mRNA level analysed by Northern-blot, following treatment of VSMC with increasing concentrations of PDGF for 2 hours. (c) Time course of 6A3-5/Osa2 protein levels investigated by Western blot. (d) Dose effect of 6A3-5/Osa2 protein levels investigated by Western blot, following VSMC treatment with increasing concentrations of PDGF for 4 hours. The results are representative of three independent experiments. Northern-blot were quantified by Quantity One tool (Bio-Rad) and normalized by 18S rRNA level. Data are presented as means \pm SEM. * : $P < .05$ versus nonstimulated control cells. The Coomassie blue-stained gel indicates equal protein loading.

Expression of 6A3-5/Osa2 and vascular phenotype in ACE inhibitor treated LH and LL rats

Four-week treatment with Perindopril (an ACE inhibitor) significantly reduced SBP in both LH (from 166 ± 3.59

to 134 ± 1.84 mmHg) and LL (from 131 ± 2.78 to 104 ± 2.39 mmHg) compared to untreated animals [16]. Interestingly, the 6A3-5/Osa2-gene expression level decreased in treated LH, but not LL, rats (Figure 10(a)). Moreover, VSMC contractile markers showed, by Q-PCR, a decrease

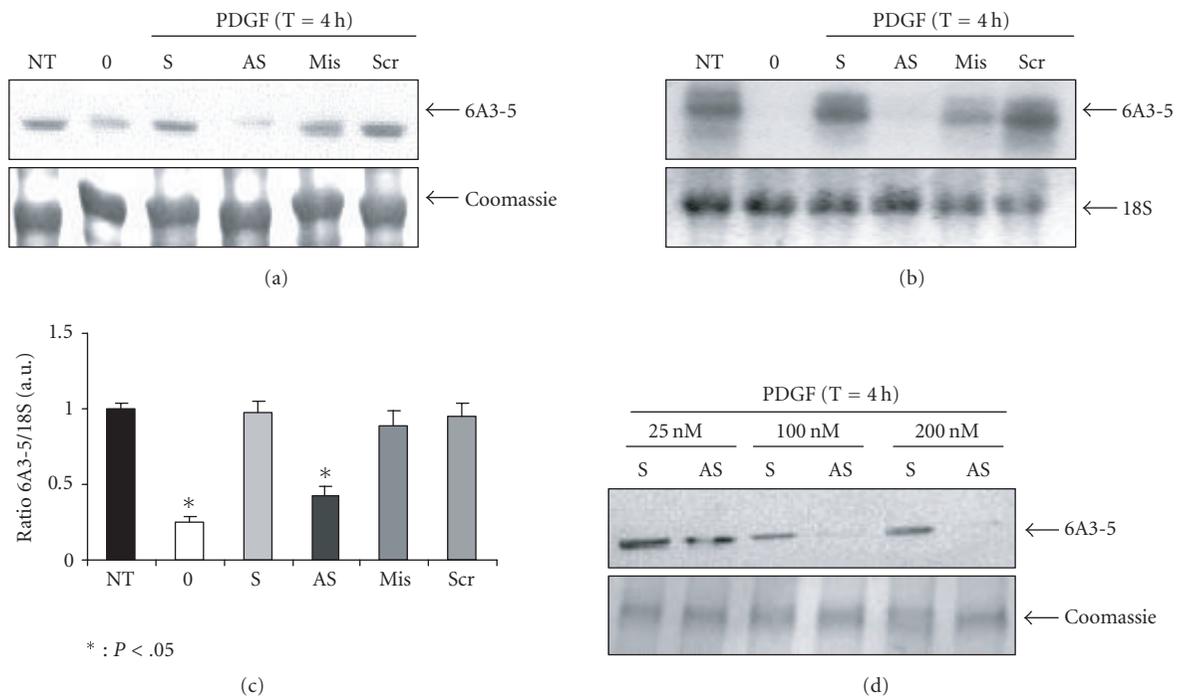


FIGURE 6: Inhibition of 6A3-5/Osa2 expression by ODN3 antisense. (a) Western blot of 6A3-5/Osa2 protein expression, following 4 hours of PDGF-BB stimulation. Serum starved VSMC (0) were treated, first, by 6A3-5/Osa2-ODN3 sense (S), antisense (AS), mismatched (Mis), scrambled (Scr), or vehicle (NT) at 200 nM. (b) Northern blot of 6A3-5/Osa2 mRNA expression, following 4 hours of PDGF-BB stimulation. (c) Quantification of Northern blot results, which are representative of three independent experiments. (d) Western blot of 6A3-5/Osa2 protein expression, following 4 hours of PDGF-BB stimulation. Serum starved VSMC (0) were treated, first, by 6A3-5/Osa2-ODN3 sense or antisense at different concentrations (25, 100, and 200 nM).

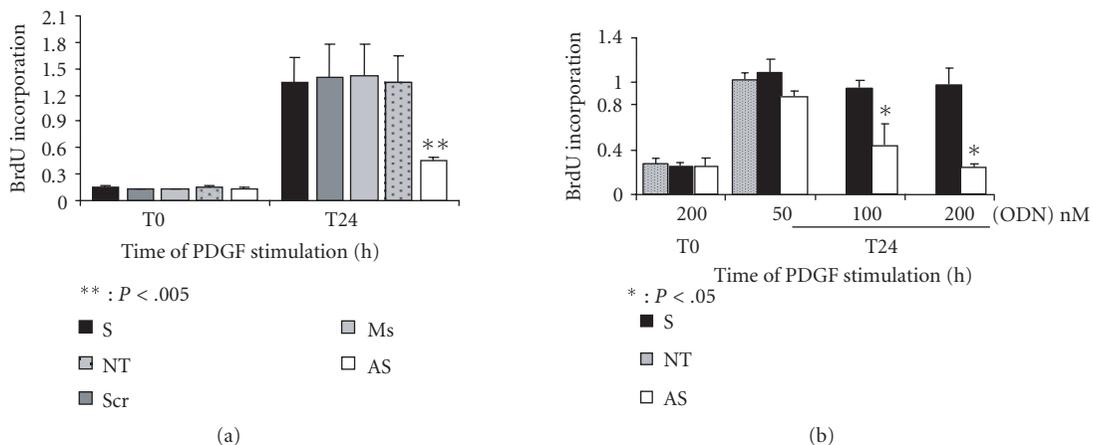


FIGURE 7: Inhibition of PDGF-stimulated VSMC proliferation by antisense ODN3. (a) Serum starved VSMC were treated by ODN3 sense, antisense, mismatched, and scrambled oligos at 200 nM followed by PDGF-BB (20 ng/ml) stimulation for 0 or 24 hours in presence of BrdU. (b) Serum starved VSMC were treated by ODN3 sense and antisense (at 50, 100, or 200 nM) following 0 and 24 hours of PDGF-BB (20 ng/ml) stimulation. Untreated VSMC are used as controls of proliferation rate. The results are representative of four independent experiments. Data are presented as means \pm SEM. * : $P < .05$ versus nontransfected cells (NT).

in calponin and SM22- α in both LH and LL animals (Figures 10(b), 10(c)). However, Vessel wall media thickness in LH and LL was not affected by such a treatment (Figure 11(c)).

Expression of 6A3-5/Osa2 and vascular phenotype in AngiotensinII-perfused LH and LL rats

Perindopril treatment, of the 2 strains, was followed by chronic perfusion of AngII which showed, over a period

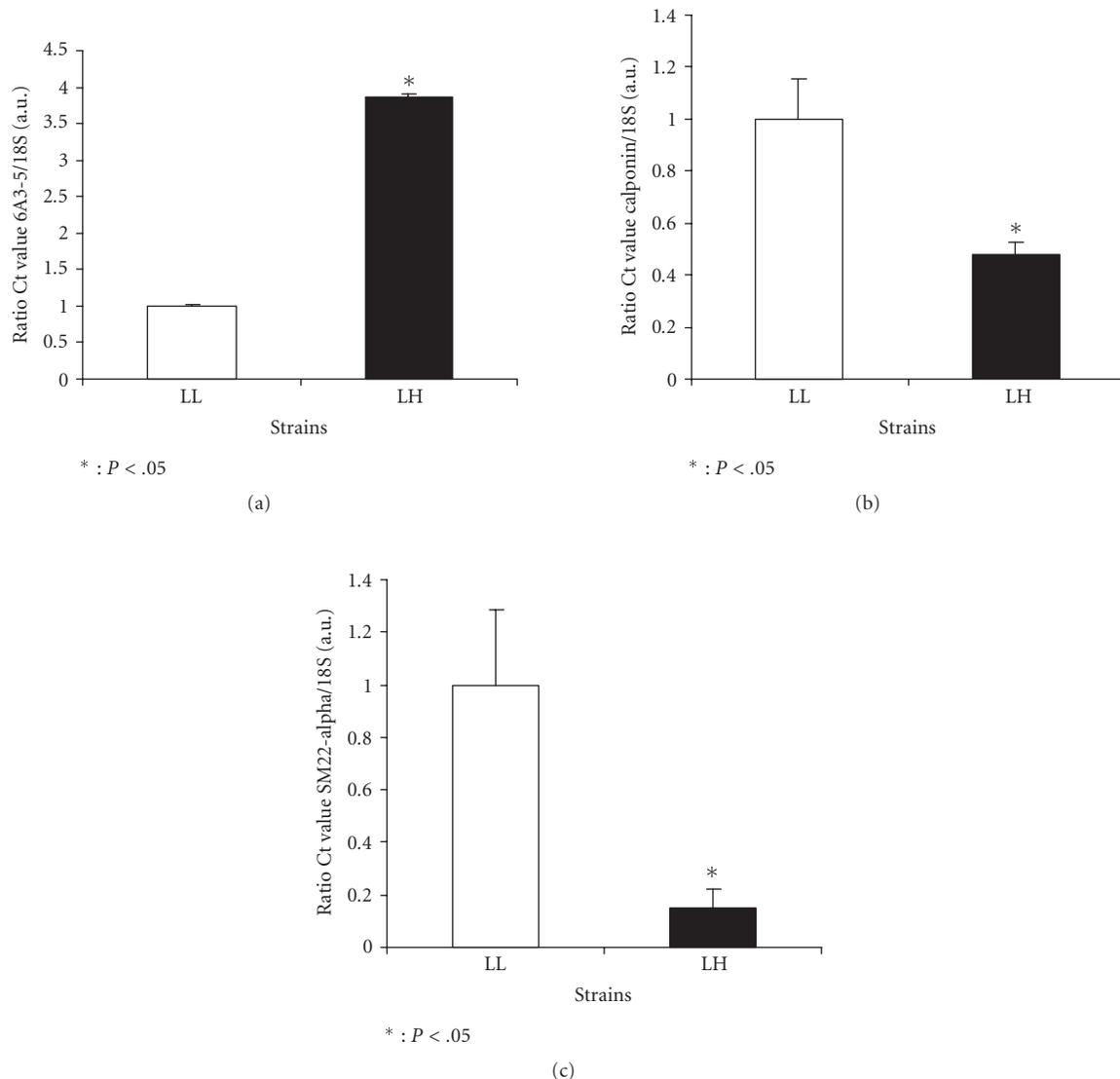


FIGURE 8: Expression of 6A3-5/Osa2 and vascular phenotype in LH versus LL rats. (a) 6A3-5/Osa2 aortic mRNA expression is significantly higher in hypertensive (LH) compared to normotensive (LL) rats. (b) Calponin (VSMC contractile phenotype marker) aortic mRNA gene expression is significantly reduced in LH versus LL rats. (c) SM22 alpha (VSMC contractile phenotype marker) aortic mRNA gene expression is significantly reduced in LH versus LL rats. Results are indicated as a ratio of mRNA expression in comparison to 18 S expression. Data are presented as means \pm SEM. * : $P < .05$ versus LL normotensive rats.

of 4 weeks, an increase of SBP in LH (from 134 ± 1.84 to 231 ± 5.67 mmHg) and a steady SBP (from 104 ± 2.39 to 192 ± 5.46 mmHg) in LL rats [16]. AngII induces a significant upregulation of aortic 6A3-5/Osa2 excised from hypertensive (LH) rats in comparison to their unperfused controls (Figure 10(a)). Moreover, decrease in VSMC contractile markers, closely followed the hypertrophy state of the vessel wall in these two strains (Figures 10(b), 10(c)). In contrast, aortic 6A3-5/Osa2-gene expression was not modified in normotensive (LL) rats. One should note that AngII perfusion induced a significant aortic media hypertrophy in LH (Figure 11(c)) and to a much lesser extent in LL rats in comparison to their unperfused controls (Figure 11(c)).

DISCUSSION

This study reports the cloning and the characterization of a new gene (6A3-5/Osa2) overexpressed in proliferating rat vascular smooth muscle cells. Several lines of evidence show that this new gene is an early-gene activator that may be implicated in the control of VSMC activities.

6A3-5/Osa2 protein bears a DNA binding motif called ARID and two recently described conserved motifs, OHD1 and OHD2. These functional domains define the recently described Osa family of transcription modulator. Recently, Hurlstone et al [14] cloned the human homologue of 6A3-5 and showed that the OHD2 motif is necessary for binding

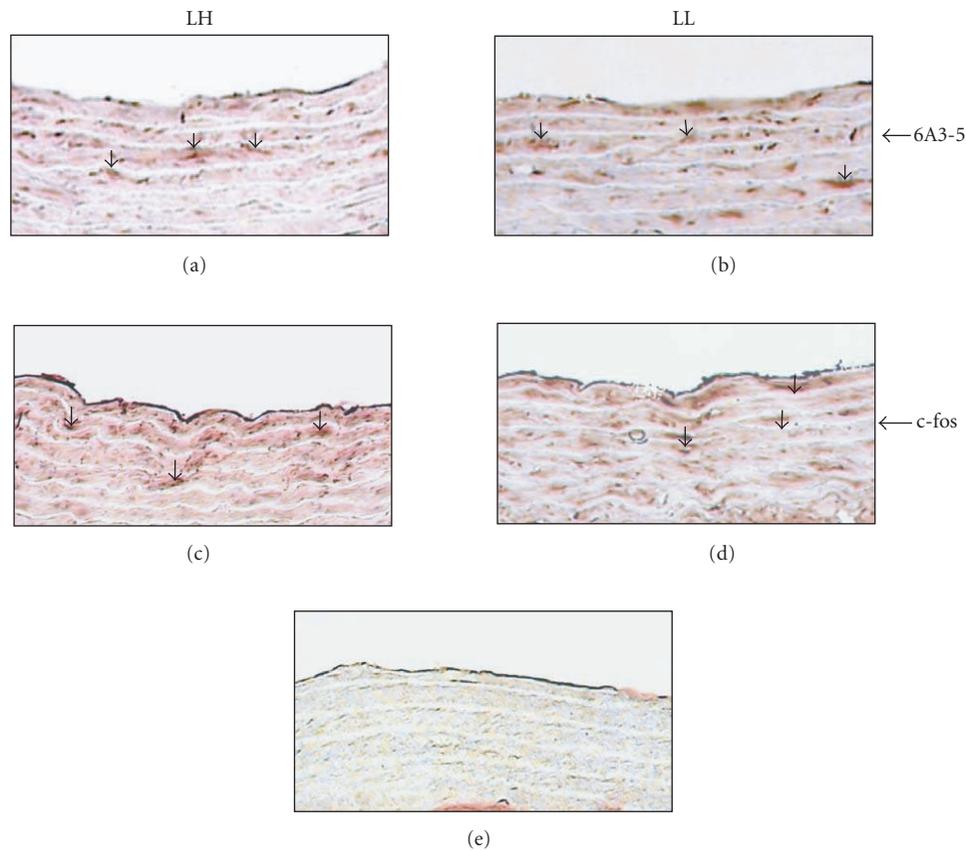


FIGURE 9: Localization of 6A3-5/Osa2 in aortas from LH and LL rats. (a) 6A3-5/Osa2 labelling is observed in SMC of the inner media from AngII-perfused LH rats (X40). (b) 6A3-5/Osa2 labelling is observed in SMC of the inner media from AngII-perfused LL rats. (c) c-fos antibody showed a similar localization to 6A3-5/Osa2 in LH rats. (d) c-fos antibody showed a similar localization to 6A3-5/Osa2 in LL rats. (e) Negative control showed no labelling. Similar localization and labelling was observed for 6A3-5/Osa2 for all tested aortas (data not shown).

BRG-1 (Brahma-related gene-1), a key catalytic component of the SWI/SNF-A chromatin remodelling complex. In contrast to other ARID proteins, Osa proteins show no sequence preference for AT rich sites. Nonetheless, work using *Drosophila* suggest that Osa proteins may participate in targeting SWI/SNF to a subset of promoters in vivo and induce the activation or repression of target gene expression. Prior to our study, no Osa protein had been described in vascular cells, and very little is known about the function of these proteins in mammals.

In this study, we have observed, in a similar way to c-fos, an early upregulation of 6A3-5/Osa2 soon after mitogenic stimulation of human or rat VSMC by PDGF-BB. Increased activity of the PDGF signalling pathway has been implicated as a contributing factor in the progression of atherosclerosis or restenosis. PDGF induces activation and phosphorylation of several cytosolic signalling molecules and nuclear transcription factors, including Egr-1 (early growth response-1), Ets-1, c-fos, and c-jun, which stimulate expression of their target genes. These data indicate that 6A3-5/Osa2 is an early PDGF-responding gene potentially implicated in VSMC proliferation. To validate this hypothesis, we generated four spe-

cific sets of ODN antisense directed against 6A3-5/Osa2. Only one of these, ODN3, is able to inhibit 6A3-5/Osa2 expression at the mRNA and the protein level in dose and sequence-dependant manner. It is interesting to note that ODN3 targets the ATG initiation site. Previous studies have demonstrated that such targeting is very effective in inhibiting gene expression by antisense phosphorothioate oligonucleotides. Indeed, ODN controls used in the present study indicated that 6A3-5/Osa2 RNA and protein depletion was due to a sequence-specific antisense effect, as neither the sense nor the scrambled or mismatched control ODNs caused 6A3-5/Osa2 depletion. Moreover, we observed no effect on p53 gene expression following Osa2 inhibition, suggesting that ODN_{AS} inhibit selectively 6A3-5 expression. We then used ODN3 in association with BrdU incorporation assays to assess the role of 6A3-5/Osa2 in VSMC proliferation. ODN3 antisense was able to significantly reduce proliferation of PDGF-stimulated VSMC in a dose and sequence-dependent manner. Recently, Watanabe et al [22] produced the first evidence that an ARID protein family member is implicated in differentiation and control of VSMC proliferation. Their study showed that overexpression of Mrf2 induces expression

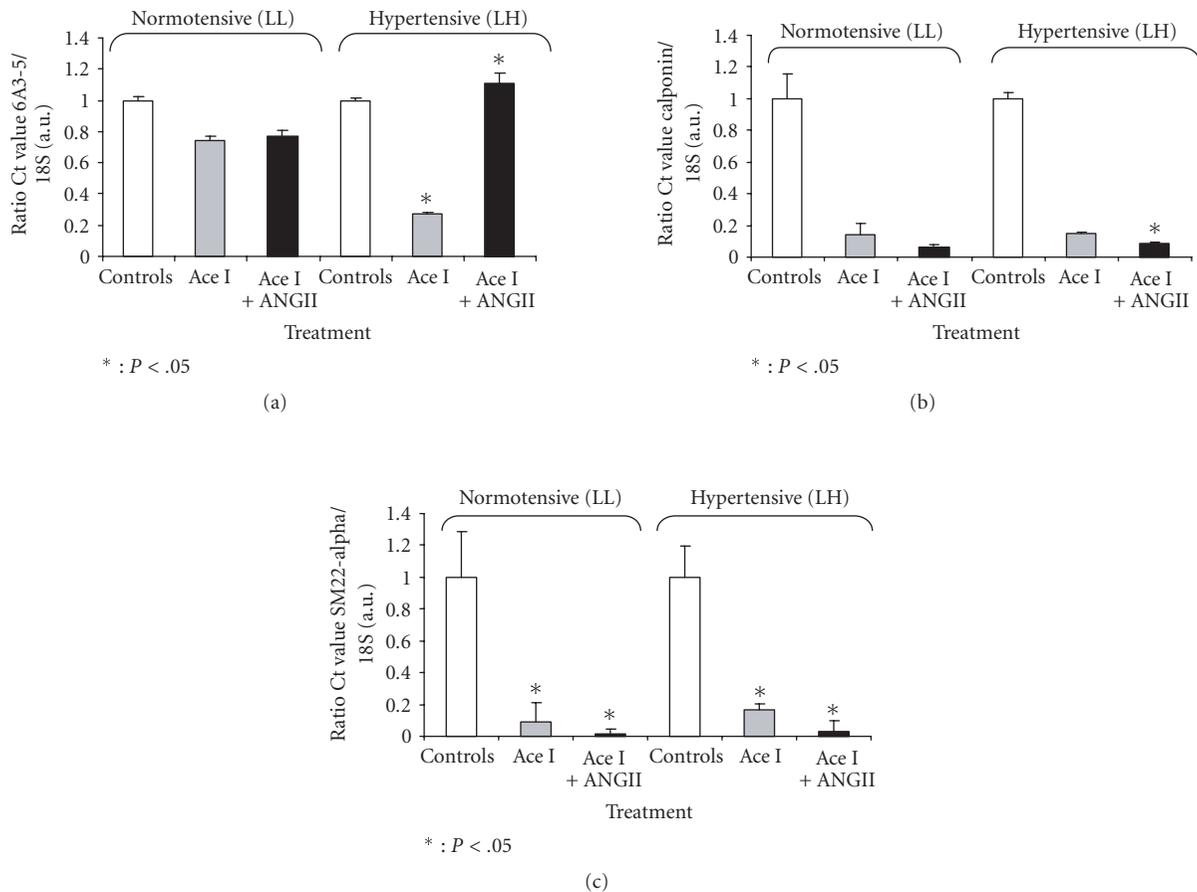


FIGURE 10: Expression of 6A3-5/Osa2 and vascular phenotype in ACE inhibitor treated LH and LL rats. (a) 6A3-5/Osa2 aortic mRNA expression in untreated LH and LL rats (controls) was compared to ACE inhibitor treated rats in the absence (Ace I) or presence of perfused ANGII (Ace I + ANGII). 6A3-5/Osa2 gene expression is downregulated by the ACE inhibitor and upregulated by exogenous AngiotensinII in LH rats. (b) Calponin gene expressions were quantified in these same animals. (c) SM22 alpha gene expressions were also quantified in these same animals. Results are indicated as a ratio of 6A3-5/Osa2 mRNA expression in comparison to 18 S expression. Data are presented as means \pm SEM. * : $P < .05$ versus controls untreated rats, for each strain.

of specific smooth muscle marker, such as alpha-actin and SM-22alpha. Interestingly, in contrast to 6A3-5/Osa2, Mrf2 retarded cellular proliferation. It is interesting to note that Mrf-2 binds a specific DNA sequence (AATA(C/T)) in contrast to Osa proteins. The apparent functional divergence in regard to cellular proliferation between the two ARID-bearing proteins could be linked to different properties of their DNA binding activities. The mechanism by which 6A3-5/Osa2 influences cell proliferation is unknown. However, human Osa2 was recently shown, to stimulate transcription as a cofactor of glucocorticoid receptor-dependent transcriptional activation in cultured mammalian cells [25]. Interestingly, glucocorticoids are known to modulate proliferation and expression of some target genes in VSMC (such as $\text{I}\kappa\text{B}$, NaKATPase, adrenomedullin). Further investigation will be necessary to investigate by which molecular mechanisms, that is, by which target genes 6A3-5/Osa2 influences VSMC proliferation.

In a similar way to PDGF, we have previously observed an early upregulation of 6A3-5/Osa2 in cultured rat VSMC

in response to AngII [15]. Several signalling responses are shared between PDGF and AngII activation. Indeed, AngII stimulation of VSMC is associated, in a similar manner to PDGF, with an upregulation of early activated genes such as c-fos and c-myc and growth factors such as PDGF and bFGF [9]. ACE inhibition by perindopril induces a reduction of c-fos and c-jun expression in response to balloon injury [26]. In vitro study has shown a link between AngII receptor and PDGF β receptor in cultured VSMC [27]. Moreover, AngII has recently been reported to transactivate the PDGF β receptor by cross-talk in stroke-prone SHR rats by comparison, Wistar-Kyoto rats their normotensive controls, did not show this effect [28]. In this study, hypertensive rats (LH) had significantly higher aortic 6A3-5/Osa2 gene expression levels in comparison to normotensive rats (LL). Moreover, while perindopril treatment reduced blood pressure in these 2 strains, it only affected 6A3-5/Osa2-gene expression in LH but not LL. Finally, exogenous AngII perfusion in the presence of ACE inhibitor increased blood pressure levels in both strains but increased 6A3-5/Osa2-gene expression only in LH

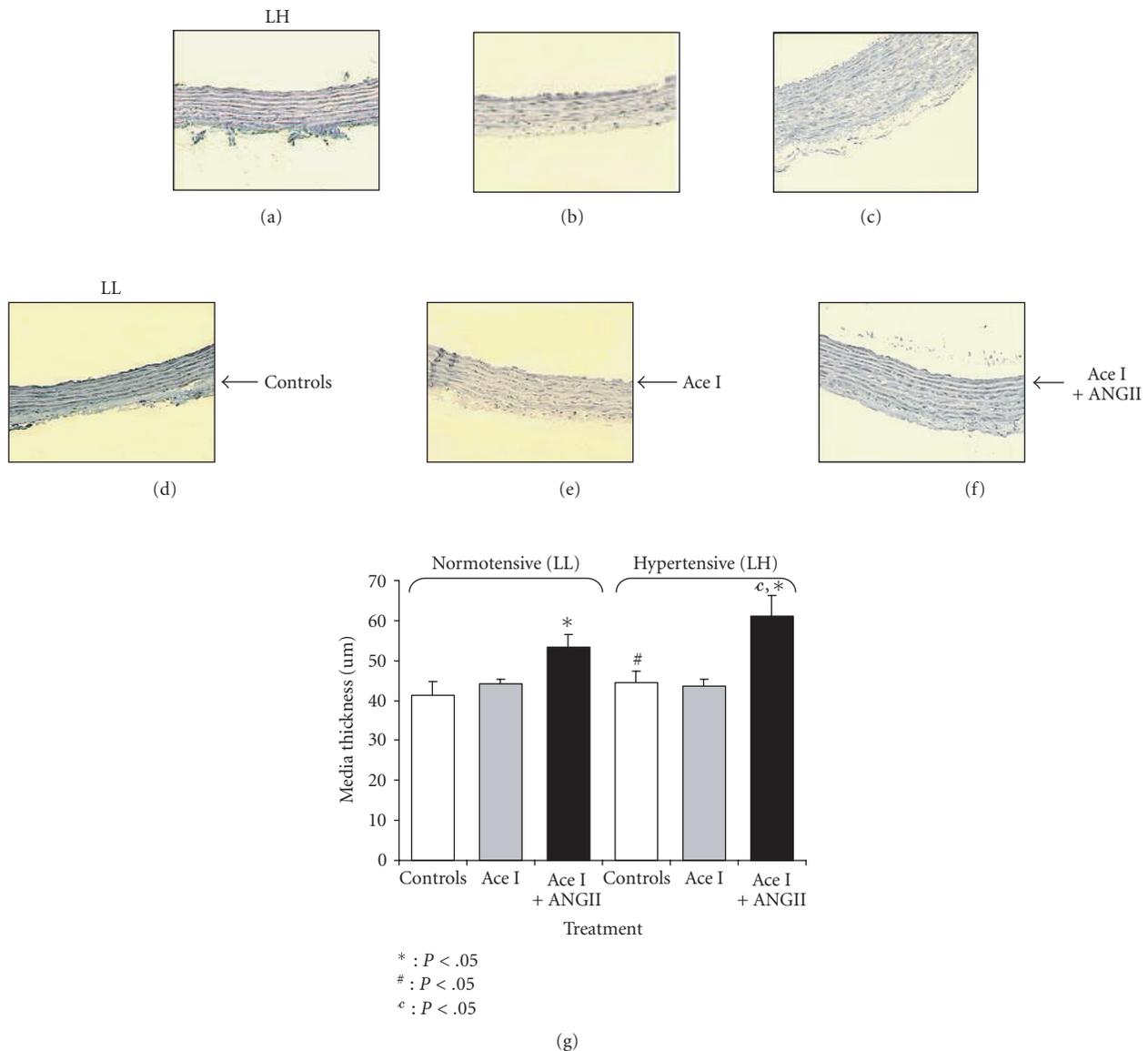


FIGURE 11: *Analysis of media hypertrophy.* Media thickness was determined, following haematoxylin/eosin staining of aorta sections. (a) Control LH rats were studied for their media thickness. (b) AceI treated LH rats were also analysed. (c) AceI and ANGII treated LH rats. (d) Control LL rats. (e) AceI treated LL rats. (f) AceI + ANGII treated LL rats. (g) Quantification of the above data is presented as means \pm SEM* : $P < .05$ versus controls for each strain. # : $P < 0.05$ versus normotensive controls rats, ^c : $P < .05$ versus AngII-perfused normotensive rats.

but not LL. Interestingly, Kim et al [28] have reported that treatment of SHR rats with perindopril significantly reduced aortic PDGF- β receptor phosphorylation and ERKinase activity which is restored by chronic (but not acute) infusion of AngII. It is known that PDGF β receptor is chronically activated in SHR compared to Wistar-Kyoto rats.

While LH rats present a higher blood pressure than LL rats, similar levels of plasma AngII were reported [29]. Interestingly, results by Lantelme et al [30], have shown that inhibition of the renin-angiotensinII system in newborn LH rats prevents the development of hypertension. It is conceivable that VSMC of LH rats are very much more sensitive to

AngII compared to LL. Such hypersensitivity has been reported for VSMC, isolated from SHR rats, which show abnormal growth in vitro with accelerated entry into S phase of cell cycle and increased cdk2 activity in comparison to VSMC from Wistar-Kyoto rats [31]. Aortic gene expression of 6A3-5/Osa2 is significantly increased in LH compared to LL rats. Such enhanced expression of 6A3-5/Osa2 gene in LH rats may be linked to the potential hypersensitivity of the VSMC that not only results in increased blood pressure but modified phenotype gene markers and media hypertrophy. On treatment with an ACE inhibitor, LH rats show a significant reduction in aortic 6A3-5/Osa2 expression, not

observed in LL rats, that is presumably due to the hypersensitivity of the VSMC to AngII. Chronic perfusion of AngII, in the presence of an ACE inhibitor, induces a significant increase in 6A3-5/Osa2 expression in LH but not in LL rats. Sabri et al [32], have shown that AngII perfusion induces a reversion of VSMC to an immature phenotype. Similarly to AngII, PDGF under in vitro conditions induces suppression of smooth muscle-specific gene (α -actin and SM22alpha) through activation of Pi3K/Akt signalling pathways and sub-cellular redistribution of serum response factor [33]. One should also note that higher glucocorticoid plasma levels are observed in LH strains in response to AngII [16]. As previously indicated, 6A3-5/Osa2 has been implicated as a cofactor of glucocorticoid receptor-dependent transcription [25]. The overall data in this study strongly suggests that the potential hypersensitivity of VSMC, in LH rats, not only controls blood pressure levels but also 6A3-5/Osa2 expression, gene markers of VSMC phenotype, and media hypertrophy.

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