THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 7, Issue of February 13, pp. 5314–5322, 2004

Printed in U.S.A.

The *VCAM-1* Gene That Encodes the Vascular Cell Adhesion Molecule Is a Target of the Sry-related High Mobility Group Box Gene, *Sox18**

Received for publication, August 4, 2003, and in revised form, October 28, 2003
Published, JBC Papers in Press, November 21, 2003, DOI 10.1074/jbc.M308512200

Brett M. Hosking, S.-C. Mary Wang, Meredith Downes, Peter Koopman‡, and George E. O. Muscat§

From the Institute for Molecular Bioscience, Queensland Biosciences Precinct, University of Queensland, Brisbane, Queensland 4072, Australia

VCAM-1 (vascular cell adhesion molecule-1) and Sox18 are involved in vascular development. VCAM-1 is an important adhesion molecule that is expressed on endothelial cells and has a critical role in endothelial activation, inflammation, lymphatic pathophysiology, and atherogenesis. The *Sry*-related high mobility group box factor Sox18 has previously been implicated in endothelial pathologies. Mutations in human and mouse Sox18 leads to hypotrichosis and lymphedema. Furthermore, both Sox18 and VCAM-1 have very similar spatio-temporal patterns of expression, which is suggestive of cross-talk. We use biochemical techniques, cell culture systems, and the *ragged opossum* (*RaOP*) mouse model with a naturally occurring mutation in *Sox18* to demonstrate that VCAM-1 is an important target of Sox18. Transfection, site-specific mutagenesis, and gel shift analyses demonstrated that Sox18 directly targeted and *trans*-activated VCAM-1 expression. Importantly, the naturally occurring Sox18 mutant attenuates the expression and activation of VCAM-1 *in vitro*. Furthermore, *in vivo* quantitation of VCAM-1 mRNA levels in wild type and *RaOP* mice demonstrates that *RaOP* animals show a dramatic and significant reduction in VCAM-1 mRNA expression in lung, skin, and skeletal muscle. Our observation that the *VCAM-1* gene is an important target of SOX18 provides the first molecular insights into the vascular abnormalities in the mouse mutant *ragged* and the human hypotrichosis-lymphedema-telangiectasia disorder.

The formation of blood vessels occurs through two distinct mechanisms, vasculogenesis and angiogenesis (1). Vasculogenesis leads to the vascularization of the endodermally derived organs such as lung, intestine, spleen, liver, and stomach, whereas angiogenesis involves the vascularization of the organs of mesodermal and ectodermal origin such as the limbs, kidney, and the brain. It also gives rise to the intersomitic and vertebral arteries (2).

We have recently implicated Sox18 in vascular development (10). Sox18 is a member of the Sry-related HMG[1] box-containing (Sox) family of transcription factors. SOX proteins bind to DNA in a sequence-specific manner via the HMG domain, with all the proteins characterized to date binding to the heptameric motif (A/T)(A/T)CAA(A/T)G (11). We have shown previously that Sox18 binds to the consensus sequence AACAAAG and *trans*-activates a heterologous promoter containing this element (12).

The Sox family displays both overlapping and distinct spatio-temporal expression patterns during embryogenesis and development. Aberrant Sox expression, mutation, or disruption leads to a number of diseases; for example, *Sry* and *Sox9* are involved in sex reversal (13, 14).

The situation is similar for Sox18. *In situ* analysis of *Sox18* has demonstrated expression in the mesenchyme underlying the developing hair follicle, in the presumptive heart, and in the developing vasculature (10). The naturally occurring mouse mutant *ragged* (*Ra*) of which there are four allelic variants, *Ra*, *ragged Jackson* (*RaJ*), *ragged-like* (*Ragl*) and *RaOP*, all contain mutations in *Sox18* (15). All these mutants display defects in hair and skin development. However, most life threatening is the generalized edema suffered by these animals (16), which is probably due to lymphatic aberrations (17–19).

Recently, a report has been published describing the investigation of several mutations in SOX18 and the hypotrichosis-lymphedema-telangiectasia (HLT) disorder in humans (20). Patients present with early onset alopecia of the scalp and lymphedema. The most severe lymphatic abnormality presented was non-immune hydrops fetalis (of unknown etiology). Telangiectasia was present in only in some of the patients studied, as were other anomalies such as thinness and transparency of the skin, hydrocele, and cutaneous papular vascular lesions.

One of the major functions for the blood and lymphatic vascular system is to provide efficient access for leukocytes and other immune system molecules to all tissues of larger animals. The immune system responds to damage or illness via the accumulation of leukocytes, leading to a localized inflammation in the diseased area. This inflammatory response is necessarily tightly coordinated, as the lack of control can itself lead to various diseases, for example arthritis, psoriasis, multiple sclerosis, asthma, atherosclerosis, and allergy (21–24). Of the multitude of proteins involved in the immune system response, the cell adhesion molecules play a major role in mediating immune function and inflammation (25).

Vascular cell adhesion molecule-1 (VCAM-1), a member of

* This project was supported by a grant from the National Health and Medical Research Council of Australia. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ An Australian Research Council Professorial Research Fellow.

§ A National Health and Medical Research Council (NHMRC) Principal Research Fellow and to whom correspondence should be addressed. Fax: 61-7-3346-2101; E-mail: g.muscat@imb.uq.edu.au.

[1] The abbreviations used are: HMG, high mobility group; Sox, Sry-related HMG box-containing (protein); VCAM-1, vascular cell adhesion molecule-1; IRF-2, interferon regulatory factor-2; *Ra*, *ragged*; *RaJ*, *ragged Jackson*; *Ragl*, *ragged-like*; *RaOP*, *ragged opossum*; RT, reverse transcription; EMSA, electrophoretic mobility shift assay; GST, glutathione *S*-transferase; aa, amino acids.

the immunoglobin gene superfamily of cell adhesion molecules, is expressed on the cell surface of activated endothelia, in the skin (26, 27), and in developing muscle and the lung (28–30). VCAM-1 recognizes the integrin receptors $\alpha_4\beta_1$ (31) and $\alpha_4\beta_7$ (32, 33) present on monocytes, eosinophils, and lymphocytes, whereas VCAM-1-deficient embryos die *in utero* due to abnormalities in chorio-allantoic fusion (34). *In vivo* animal studies demonstrate that attenuated VCAM-1 function in mice provided protection against atherosclerosis. For example, mice with reduced (not ablated) VCAM-1 expression and function crossed with low density lipoprotein receptor $LDLR^{-}/^{-}$ mice prone to atherosclerosis produce animals resistant to atherogenesis (35).

Much of the previous research has focused on the control of VCAM-1 expression via extracellular signals. For example, lipopolysaccharide and cytokines such as interleukin-4 (IL-4), tumor necrosis factor-α (TNF-α) (36, 37), interferon-γ (INF-γ), transforming growth factor-β1 (TGF-β1) (38), granulocyte-macrophage colony-stimulating factor (GM-CSF) (39), and vascular endothelial growth factor (VEGF) (40) cause an increase in the level of expression of VCAM-1 in endothelia as well as in other cell types, whereas angiopoietin 1 (ang1) can repress the activation of VCAM-1 by vascular endothelial growth factor (41).

In contrast, transcriptional regulation of VCAM-1 is less well understood. Most notable is the role of interferon regulatory factor-2 (IRF-2) in the control of VCAM-1 expression in muscle (42).

Sox factors act as critical regulators of organ ontogeny via the modulation of expression of particular target genes. Surprisingly, relatively few target genes for this family of transcription factors have been described in the literature (11). Therefore, to elucidate the function of Sox18 in the blood and lymphatic vascular system, the major site of expression and of abnormality in the *ragged* animal, it is important to find target genes in this organ.

Our study demonstrates that native Sox18 (and not the mutant *ragged* form) is able to induce the activity of the VCAM-1 promoter. In biochemical assays we identified and characterized three Sox18 binding sites. However, we demonstrated that only the SoxB site at −715 is necessary for VCAM-1 *trans*-activation. *In vivo* validation of this data was obtained from the *RaOP* animals that have very significantly reduced levels (8–40-fold) of VCAM-1 expression (and not other adhesion molecules, including ICAM-1 and ICAM-2, JCAM, NCAM-1, and PECAM-1).

MATERIALS AND METHODS

RNA and cDNA Preparation—RNA for realtime PCR was isolated from organs of *RaOP* and wild-type adult male siblings as described previously (45), with the exception that the lyophilized RNA was purified by processing ~100 μg through a Qiagen RNeasy mini column. During this purification process the RNA was DNase-treated. Quantitation of the purified RNA was carried out as described previously (45). First strand cDNA synthesis was carried out using 5 μg of purified RNA and primed with oligo$(dT)_{18}$, using the Superscript III enzyme and the supplied manufacturer's protocol.

Real Time PCR—Target cDNA levels were quantitated by real time RT-PCR using an ABI Prism™ 7700 Sequence Detector system utilizing SYBR Green I (Molecular Probes, Eugene OR; used at 0.8×) as a nonspecific PCR product fluorescence label. Quantitation was >45 cycles of 95 °C for 15 s and 60 °C for 1 min two-step thermal cycling preceded by an initial 95 °C for 2 min for activation of 0.75 units of Platinum® TaqDNA polymerase (Invitrogen). The 25-μl reaction also contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 5 mM $MgCl_2$, 200 μM each of dGTP, dATP, and dCTP, 400 μM dUTP, 0.5 units of uracil-*N*-glycosylase, 500 nM ROX reference dye (Invitrogen), and 200 nM each forward and reverse primers. *Mus musculus* primer sequences for VCAM-1 are as follows: forward, 5′-TGACAAGTCCCCATCGTTGA-3′; reverse, 5′-ACCTCGCGACGGCATAATT-3′.

Promoter Construct Generation—The sequence of the murine VCAM-1 promoter has been published elsewhere (46) and is available on NCBI (accession number U42327). Mouse genomic DNA (C57BL/6) was a gift from J. Rowland, and 10 ng was used in the outer PCR amplification to generate the VCAM-1 promoter. Nested PCR was necessary for amplification of the 1895-bp promoter that has been published previously. Outer primers were constructed at the published upstream limit of murine VCAM-1 promoter at −1895 bp and within the first exon at 19 bp. The outer PCR was performed with 100 ng of each primer (−1895, 5′-GCCGGTACCGATCTACATAGCCACG-GAGAG-3′; and 19, 5′-CGACCATCTTCACAGGCATTT-3′), 1.25 units of *Pfu*®, and 0.2 mM dNTPs in a final volume of 50 μl containing the supplied buffer (Promega). Hot start PCR was performed at 95 °C for 5 min followed by 35 cycles of 95 °C for 1 min, 60 °C for 1 min, and 68 °C for 4 min. The inner primers were constructed such that the 5′-primer at −1889 contained the restriction enzyme site KpnI (−1887, 5′-CGG-GGTACCATAGCCACGGAGAGTTCTT-3′), whereas, the 3′-primer had the restriction enzyme site XhoI (−1, 5′-GCCCTCGAGTTCAAGTCTC-TGCTTCAAAGCC-3′). 1 μl of the outer PCR was combined with buffer B and 0.25 units of polymerase mix from the Fail-Safe PCR system (Epicenter) and 100 ng of each inner primer in a final volume of 10 μl. The VCAM-1 inner PCR profile was the same as that used for the outer PCR.

The product was digested with KpnI and XhoI, inserted into pGL2-Basic (pGL2B-Promega), and sequenced completely via automated sequencing using the ABI system and reagents. This clone was then used as the parental plasmid to generate all other sub-clones. Deletion clones were generated either by restriction digests or PCR amplification. VC1219 was generated by restriction digest with PstI and KpnI, and the vector plus the remaining insert were then blunt-ended with Klenow (New England Biolabs) and religated. Both VC754 and 504 were generated via PCR amplification with the primers 5′-GACTTCCTGT-CATCCAGCAATGGGTCAAA-3′ and 5′-CGGGGTACCTTTGTTGAAA-GAG-3′, respectively. The 3′-primer was the inner primer −1 from the initial nested PCR. The PCR profile was essentially the same as that done for the nested PCR, with the exception that the annealing temperature was 55 °C. Site-directed mutagenesis of the VCAM-1 promoter was undertaken via the QuikChange® II kit from Stratagene and carried out according to the manufacturer's protocol. The primers used for the −1569 mutation are 5′-ATGACATGACATCATTGAGGTC-CTCTAG-3′ and 5′-CTAGAGGACCTCAATGATGTCATGTCAT-3′. The primers used for the −715 mutation are 5′-GCTGGGGCATCATCAAA-CAAAA-3′ and 5′-TTTTGTTTGATGATGCCCCAG-3′.

Cell Culture and Transient Transfections—COS-1 (simian fibroblast) and SVEC4-10 (mouse high venule endothelial) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum in 6% CO_2. C_2C_{12} (mouse skeletal muscle) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 20% fetal calf serum in 6% CO_2. Cells for transfection were grown in 24-well dishes to 50–60% confluence before being transiently transfected with 2 μg of the reporter plasmid and 1 μg of the expression plasmids in 0.5 ml of Dulbecco's modified Eagle's medium containing 10% fetal calf serum by a liposome-mediated procedure. Briefly, SVEC4-10 cells were transfected using *N*-[1-(2,3-dioleoyloxy)]-*N,N,N*-trimethylammonium propane methylsulfate (DOTAP) (Roche Molecular Biochemicals) in quantities 6-fold (v/w) the total amount of DNA, whereas COS-1 and C_2C_{12} cells were transfected using 15 μl of DOTAP and 10 μl of 1,3-dioleoyloxy-2-(6-carboxyspermyl)-propylamid (DOSPER) (Roche Molecular Biochemicals). 24 h post-transfection the medium was replaced, and the cells were grown for a further 24–48 h. Each experiment represented at least two sets of independent quadruplicates to overcome the variability inherent in transfections. Cells were harvested and assayed for luciferase activity as described previously (47).

Electrophoretic Mobility Shift Assay (EMSA)—We have described previously the cloning and protein expression of a murine Sox 18 fusion construct with glutathione-*S*-transferase (GST) using the pGEX-1 bacterial expression vector (12). Briefly, *Escherichia coli* DH5a cells containing this vector were induced for 1–2 h with 0.5 mM isopropyl thiogalactoside after the cells had grown to an A_{600} of 0.6. The pelleted cells were sonicated, and the cleared lysate containing the fusion protein was loaded onto glutathione-agarose columns in Dignam buffer C (containing protease inhibitors) (47). After extensive column washing, the fusion protein was eluted with Dignam buffer C supplemented with 5 mM reduced glutathione.

Probes used in all EMSAs were annealed and then radiolabeled using γ-^{32}P and T4 polynucleotide kinase (PNK). The oligonucleotide sequences used as probes are as follows: −1569, 5′-TTTTATGACATGA-CattgttGAGGTCCTC-3′ (top strand) and 5′-GAGGACCTCaacaatGTC-

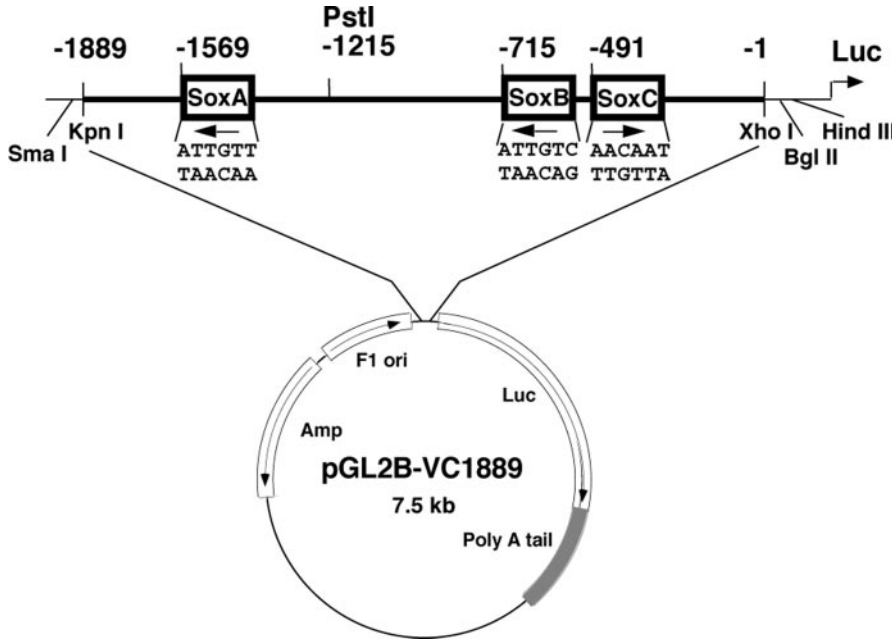

FIG. 1. **Schematic representation of the murine VCAM-1 promoter and the positions of elements important for this study.** A schematic diagram of the full-length VCAM-1 promoter construct cloned and used in this investigation is shown. Of particular note are the three putative Sox binding sites displayed as *bold boxes* and denoted as SoxA, SoxB, and SoxC. The core heptameric Sox sequence is displayed below each box with an *arrow* to indicate the direction of each binding site as compared with the direction of transcription. All numbers designate important elements or clones referred to in the text. *Luc* refers to the luciferase reporter gene, *F1 ori* denotes the origin of replication of filamentous phage for generation of single-stranded DNA, and *Amp* represents the ampicillin-resistance gene.

ATGTCATAAAA-3′ (bottom strand); −715, 5′-GGCTGGGGCattgtcAA-ACAAAAG-3′ (top strand) and 5′-CTTTTGTTTgacaatGCCCCAGCC-3′ (bottom strand); −491, 5′-GAAAGAGaacaatTTTTATTTTTTAAATTG-CAAATGCATTTCTT-3′ (top strand) and 5′-AAGAAATGCATTTGCAA-TTTAAAAAATAAAAattgttCTCTTTC-3′ (bottom strand).

The bases in lowercase letters represent the putative Sox binding sites. All EMSA experiments were carried out in a total of 20 μl in Dignam Buffer C containing 1–2 ng of T4 polynucleotide kinase-labeled probe and 2 μg of the purified, bacterially expressed GST-Sox 18. The assays were incubated at room temperature for 20 min and electrophoresed through a 6% (polyacrylamide/bisacrylamide; 20:1) gel in 80 mM Tris borate and 2 mM EDTA. Gels were briefly soaked in 10% acetic acid, dried, and autoradiographed.

Competition EMSAs were carried out basically as above with the exception that unlabeled probe was added to the reaction in 20–80-fold molar excess as compared with the labeled probe. Both the probe and an unlabeled competitor were added to the reaction at the same time and then incubated and electrophoresed as before. For these experiments, the unlabeled competitor in each reaction was the same double-stranded oligonucleotide as the probe used in that reaction.

RESULTS

Cell-specific Transcription of the Mouse VCAM-1 Is Regulated by the 5′-Upstream Flanking Sequences—The naturally occurring Sox18 mutations in mice and humans (15, 20) display defects in hair and skin development. However, the most life threatening is the generalized edema caused by lymphatic vascular dysfunction (17–19). Hence, we were particularly interested in the identification of Sox18 target genes that play a role in lymphatic function and thus may allow us to better understand the molecular basis of the *ragged* phenotype.

We hypothesized that VCAM-1 was regulated by SOX18. Therefore, to test this supposition we examined the ability of VCAM-1 promoter sequences to direct the expression of the *LUC* gene in muscle, endothelial, and fibroblast cells. For this purpose, we designed primers to amplify the complete published murine VCAM-1 sequence (46) and cloned the sequenced amplified product into the promoterless pGL2-Basic luciferase reporter vector. This plasmid was denoted as PGL2-VC1889 and encompasses 1889 bp immediately upstream of the murine VCAM-1 translation start codon (Fig. 1). To test if Sox18 could *trans*-activate the VCAM-1 promoter, we transfected it into fibroblasts and endothelial and skeletal muscle cell lines (Fig. 2). Transfection of pGL2-VC1889 into COS-1 fibroblasts and SVEC4-10 high venule endothelial cell lines demonstrated that the upstream promoter sequences of the VCAM-1 gene confer

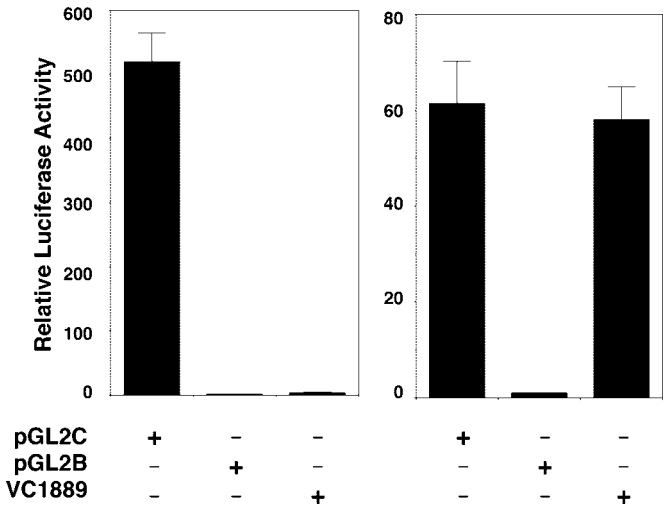

FIG. 2. **VCAM-1 promoter regulates transcription in a cell/tissue-specific manner.** The activity of the full-length VCAM-1 promoter in COS-1 fibroblast (*left*) and SVEC4-10 endothelial (*right*) cells is depicted. pGL2 control (*pGL2C*), driven by the promiscuous SV40 enhancer and early promoter, was used as a control for transfection efficiency and as a comparison for the level of cell-specific activity of the VCAM-1 promoter. pGL2-Basic (*pGL2*) is the empty luciferase vector, whereas VC1889 refers to 1889 bp of the murine VCAM-1 promoter cloned into pGL2B. 2 μg of each plasmid was transfected in all cell lines using a liposome-mediated procedure. Results are expressed as mean ± S.D. of two sets of independent quadruplicates.

high level expression in a cell-specific manner. For example, pGL2-VC1889 activity in COS-1 fibroblasts is ~ 5-fold greater than that of pGL2-Basic and 100-fold less than that of the constitutively active SV40 promoter. In contrast, in endothelial SVEC cells pGL2-VC1889 activity is ~50-fold greater than that of pGL2-Basic and is similar in activity to the very efficient SV40 promoter. This is consistent with our previous study that reported a high level of Sox18 expression in the nuclei of SVEC4-10 (48). Similarly, when the VCAM promoter was transfected into proliferating C2C12 myoblasts, the VCAM-1 promoter activity was ~50-fold greater than that of pGL2-Basic (data not shown), as has been reported previously (44). In conclusion, the efficient cell specific expression of VCAM-1 is consistent with the *in vivo* expression profile of VCAM-1.

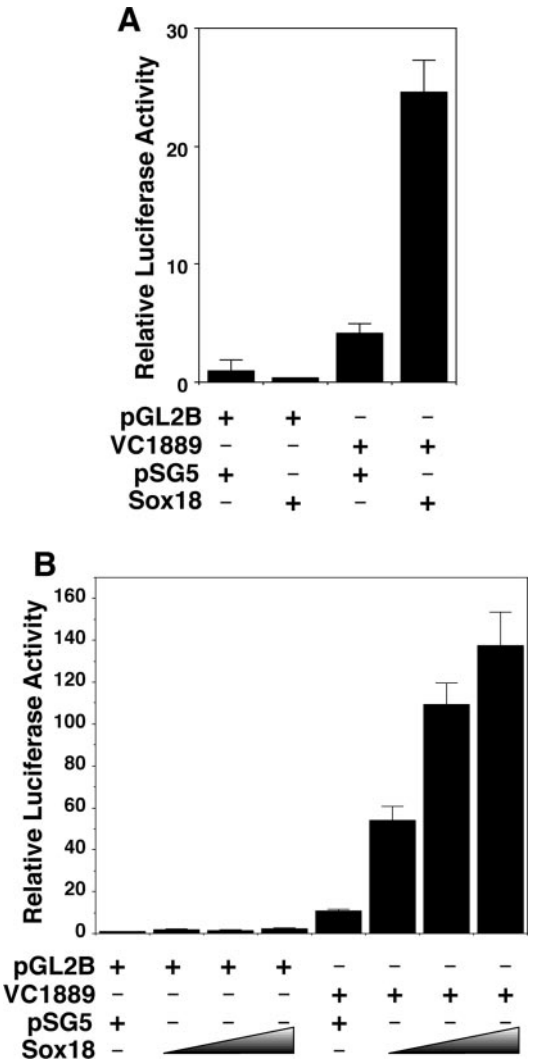

FIG. 3. **Sox18 activates the VCAM-1 promoter.** *A*, co-transfection of COS-1 cells with control vectors (*pSG5*, an expression vector and *pGL2B*, a luciferase reporter vector) and with pSG5 containing Sox18 (*Sox18*) and pGL2B containing 1889 bp of the proximal mVCAM-1 promoter (*VC1889*). *B*, co-transfection of increasing amounts of *Sox18* leads to the dose-dependent expression of the mVCAM-1 reporter (*VC1889*). 1, 2, and 3 μg of Sox18 expression vector were transfected in *lanes 2–4*, *6* and 7, and 8, respectively (counting from the *left*). The results are expressed as the mean ± S.D. of at least two independent quadruplicates.

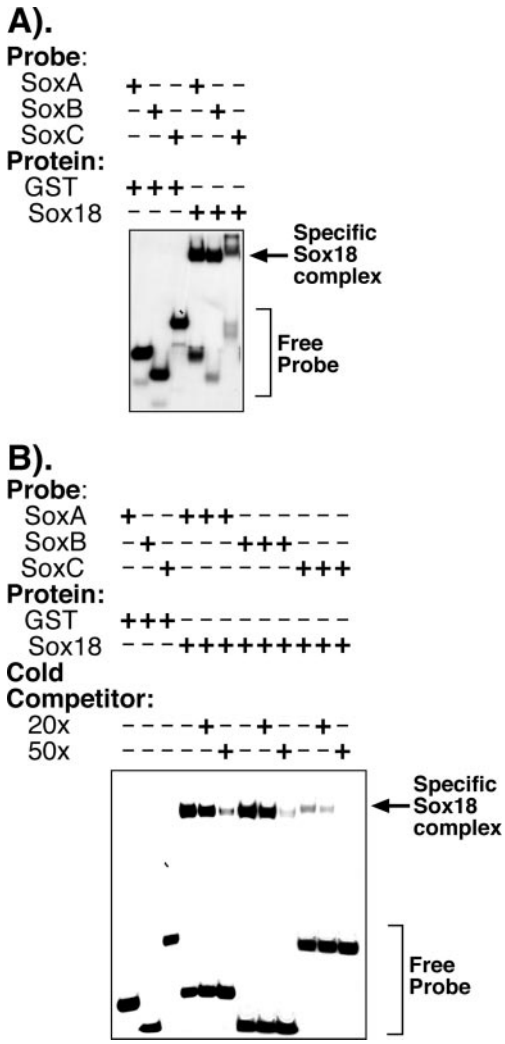

FIG. 4. **Sox18 binds to multiple sites in the VCAM-1 promoter.** *A*, a bacterially produced GST-Sox18 fusion protein binds strongly as a single band to all putative Sox sites in the VCAM-1 promoter (*arrow*). *Lanes 1–3* (counting from the *left*) delineate that the GST protein alone can not contribute to the binding of these oligonucleotides. All oligos were ^{32}P-radiolabeled, combined with GST-Sox18, and then electrophoresed on 6% polyacrylamide gels. *B*, EMSA competition assays with up to 50-fold molar excess of "cold" unlabeled oligos were used delineate the specificity of SOX18 binding to the Sox motifs.

The VCAM-1 Gene Is a Target of the Sry-related HMG Box Gene Sox18 as Shown by the Identification of Three SOX18 Binding Sites—VCAM-1 expression can be stimulated via cytokines (*e.g.* tumor necrosis factor-α) (43), mitogens (*e.g.* vascular endothelial growth factor) (49), and transcription factors (*e.g.* IRF-2 and Oct-1) (42, 43). We investigated whether SOX18, could *trans*-activate the mouse VCAM-1 promoter in COS-1 fibroblasts. Co-transfection of a Sox18 expression construct with the pGL2-VC1889 promoter resulted in a moderate 5-fold activation (Fig. 3*A*). This activation is dose-dependent, as increasing amounts of Sox18 augmented the *trans*-activity of the VCAM-1 promoter (Fig. 3*B*). Thus, we demonstrate here for the first time that Sox18 *trans*-activates the promoter of VCAM-1.

SOX and SRY proteins contain HMG domains that bind DNA in a sequence-specific manner. These proteins have been reported to bind *in vitro* and *in vivo* to sites with a core motif resembling (A/T)(A/T)CAA(A/T)G (for review, see Ref. 11). We have reported previously that SOX18 can bind and *trans*-activate a core consensus motif of AACAAAG (12). As an initial step in the investigation to examine whether SOX18 was able to bind the murine VCAM-1 promoter, we searched the sequence of the promoter using MatInspector (50) and found three potential Sox binding sites (Fig. 1). Of these, the two at −491 (SoxC) and −1569 (SoxA) contain a perfect core consensus site (AACAAT) for the binding of Sox/Sry transcription factors. The core sequence of the site at −715 (SoxB) is slightly different from the consensus, with a G at position 1 (GACAAT). Interestingly, it has been reported that SOX17 can bind this sequence from a pool of otherwise random oligonucleotides (51).

To investigate whether GST-SOX18 can interact with the putative SoxA, SoxB, and SoxC binding sites, we performed an EMSA using ^{32}P-labeled oligos that consist of the Sox binding site in addition to some surrounding sequence (see "Materials and Methods"). As seen in Fig. 4*A*, the SOX18 protein bound strongly to all three putative Sox sites. In contrast, GST protein alone could not bind to any of the three oligonucleotides used in this study. Thus, SOX18 can potentially interact with all three sites in the VCAM-1 promoter. To examine the sequence-specificity of the protein-DNA interaction, we at-

tempted to compete the binding with cold/unlabeled double-stranded oligonucleotide with the same sequence as the probe used in the reaction. Fig. 4*B* demonstrates that these sequences effectively competed for the formation of the SOX18-DNA complex. These experiments reveal that SOX18 binds in an efficient and sequence-specific manner to all three putative Sox sites.

The Sox B site at −715 bp Is Essential for trans-Activation by Sox18—In an attempt to further elucidate which sites were necessary for the *in vivo trans*-activation of VCAM-1 in fibroblast cells, we produced a series of 5′ unidirectional deletions in the mVCAM-1 promoter. Three deletion constructs were produced, namely pGL2-VC1215, pGL2-VC754, and pGL2-VC504, which contained 1215, 754, and 504 nucleotides, respectively, of 5′ flanking sequences cloned upstream of the luciferase reporter in pGL2-Basic.

By transfecting the full-length and deleted promoters in the absence/presence of the Sox18 expression vector in COS-1 cells, we could demonstrate that VC1889, VC1215, and VC754 were *trans*-activated by SOX18 (Fig. 5*A*). In contrast, deletion of the sequences from −715 to −504 completely ablated SOX18-mediated *trans*-activation. This suggested that the cognate Sox18 binding site at −715 was necessary for *trans*-activation. Moreover, the most proximal Sox binding site (−504 bp) could not independently support or mediate *trans*-activation. Interestingly, the sequences between −1889 and −715 seem to contain elements that confer repression of the activity of the VCAM-1 promoter. This repressive activity may be due to the putative octamer sites present in this region.[2]

To directly identify which of the two potential Sox sites in the VCAM-1 promoter at −1569 and −715 bp are necessary for Sox18 mediated *trans*-activation, we used site-directed mutagenesis (Fig. 6*A*). Two bases in the middle of each of the heptameric Sox sites were mutated (Fig. 6*D*). Transfection of the clone VC1889-*mSoxA* (Fig. 6, *B*, and *C*) demonstrated that the mutation of the distal SoxA binding site at −1569 did not compromise Sox18-mediated *trans*-activation of the VCAM-1 promoter. In dramatic contrast, mutation of Sox site B at −715 bp in the construct VC1889-*mSoxB*, completely abrogated the basal activity of the promoter and also ablated the Sox18-mediated *trans*-activation of the murine VCAM-1 promoter. These results, in addition to those from the deletion experiments, show that the SoxB site at −715 bp in the murine VCAM-1 promoter is necessary for *trans*-activation by Sox18.

The functional analysis correlated with the *in vitro* binding data. For example, we examined the ability of the mutant mSoxA and mSoxB motifs (Fig. 6*D*) to compete for SOX binding relative to the wild type motifs in EMSA competition assays. Fig. 6*E* demonstrates that the ability of the mutated Sox motifs to compete for SOX binding is certainly compromised relative to the native motifs. Fig. 6*F* demonstrates that even at 80-fold molar excess the mutated sequences very inefficiently compete for SOX protein binding. These experiments demonstrate that SoxB is necessary for *trans*-activation by Sox18 and that significant *trans*-activation of the VCAM-1 promoter by Sox18 is associated with efficient binding to the SoxB motif.

The ragged Alleles of SOX18 Cannot trans-Activate VCAM-1 Expression—We have reported previously that *Sox18* is mutated in all four alleles of *ragged* (*Ra*, *RaJ*, *Ragl*, and *RaOP*). *ragged* is a mouse mutant that displays hair follicle, vascular, and lymphatic abnormalities (10, 15). All four mutants consist of a missense mutation that occurs in the activation domain (aa 252–345) for *Ra* (aa 313), *RaJ* (aa 312), and *Ragl* (aa 323), whereas the *RaOP* (aa 349) mutation is 4 bp C-terminal of this

[2] B. M. Hosking, personal observation.

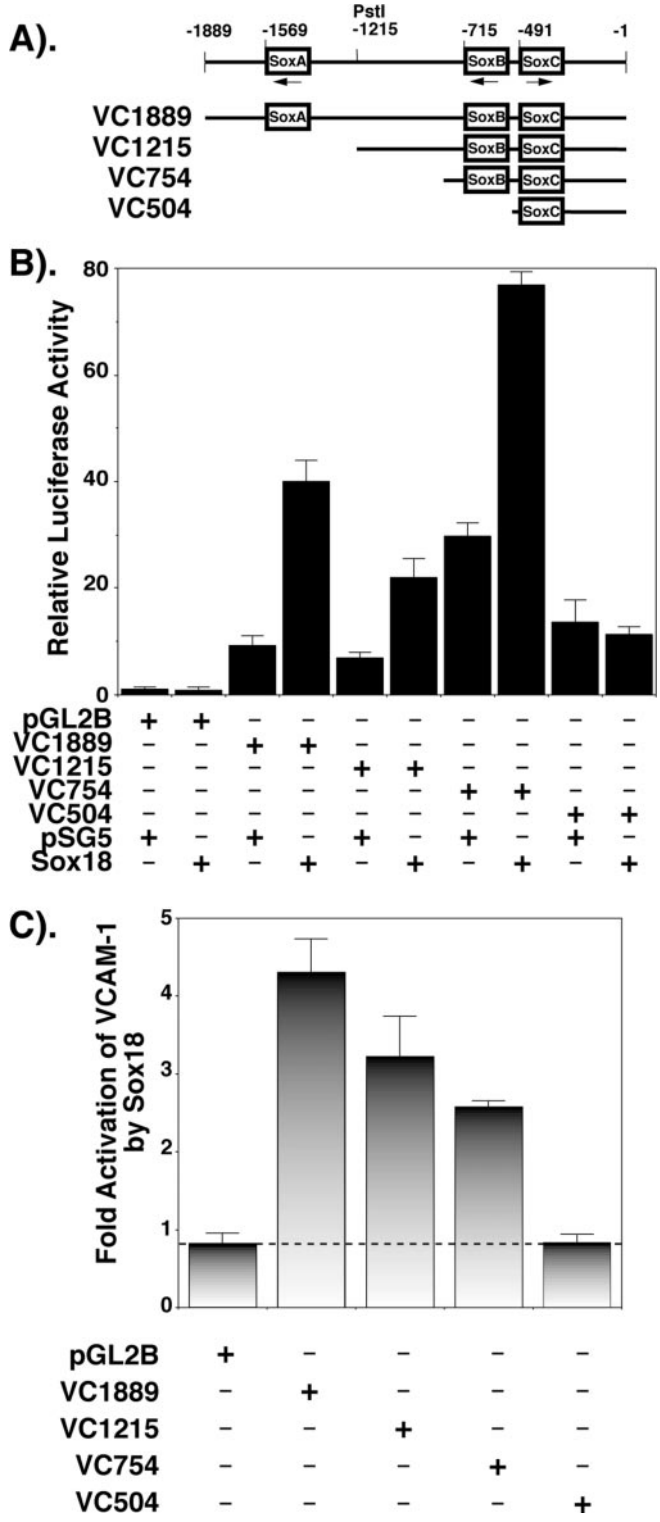

FIG. 5. **Deletion analysis defines the *cis*-acting region between −754 and −504 as important in Sox18-mediated *trans*-activation in all cell lines tested.** *A*, schematic representation of the deletion constructs. *B*, pGL2-Basic and the unidirectional deletion constructs of the VCAM-1 promoter cloned into pGL2-Basic were co-transfected into COS-1 fibroblasts with either pSG5 (empty vector) or pSG5-*Sox18*. Relative luciferase activity is compared with the luciferase activity obtained after co-transfection of pGL2-Basic with pSG5 (empty vector alone) and arbitrarily set to 1. *C*, the data from *panel B* is re-presented as the fold activation of the VCAM-1 promoter constructs by *Sox18*. Fold activation is expressed relative to the activation of the empty luciferase vector, pGL2-Basic, and co-transfected with the expression vector, pSG5-*Sox18*, which was arbitrarily set to 1. The mean luciferase fold activation values and S.D. values were derived from 2–3 independent quadruplicates.

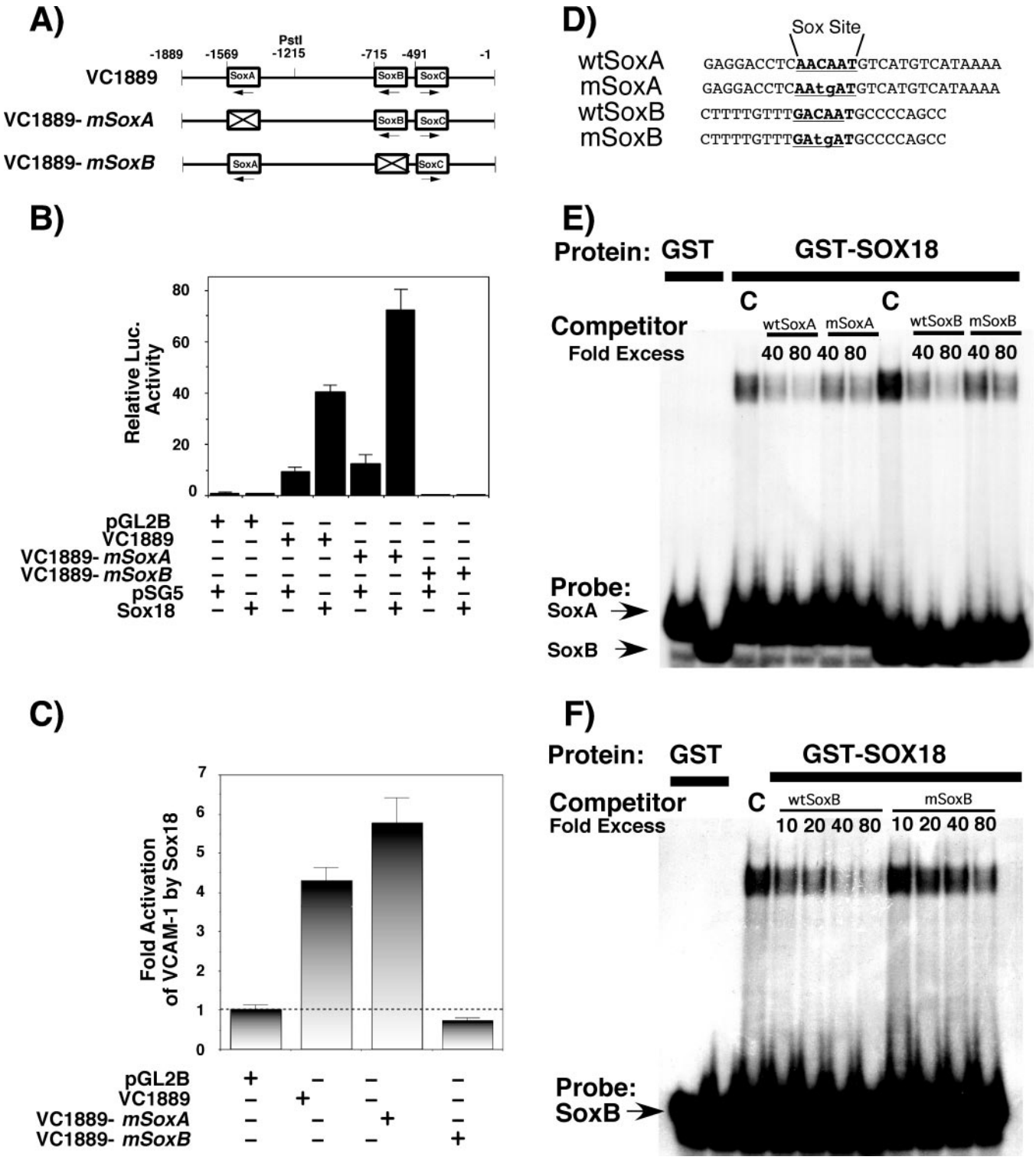

FIG. 6. **Site-directed mutation analysis identifies the Sox binding site B (−715 bp) as important in Sox18-mediated *trans*-activation of the VCAM-1 promoter in all cell lines tested.** *A*, schematic representation of the mutated constructs. The *bold boxes with crosses* represent the mutated (*m*) putative Sox binding sites. *B*, pGL2Basic and the site-directed mutations (*m*) of the VCAM-1 promoter cloned into pGL2Basic were co-transfected into COS-1 fibroblasts with pSG5 (empty vector) or pSG5-Sox18 (Sox18). Relative luciferase (*Luc.*) activity is compared with the luciferase activity obtained after transfection of pGL2Basic with the empty vector pSG5 and arbitrarily set to 1. *C*, the data from B is re-presented as the fold activation of the VCAM-1 promoter constructs by Sox18. Fold activation is expressed relative to the activation of the empty luciferase vector pGL2Basic co-transfected with the expression vector pSG5-Sox18, which was arbitrarily set to 1. The mean luciferase fold activation values and S.D. values were derived from 2–3 independent quadruplicates. *D*, schematic describing the native (*wt*) and mutated (*m*) SoxA and B motifs. *E* and *F*, EMSA competition assays with 20–80-fold molar excess, demonstrating that the mutated oligos are unable to efficiently compete for SOX18 binding. *wt*, wild type.

domain. After the frameshift mutation, all alleles encode nonsense protein until it truncates at aa 435 (15). In an effort to gain a better understanding of the molecular events occurring at the VCAM-1 promoter, we investigated the effect of the *ragged* constructs in co-transfection with the VCAM-1 promoter.

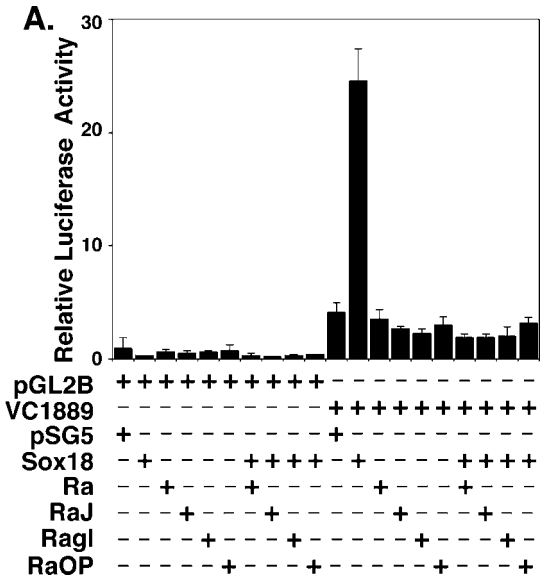

FIG. 7. **The ragged SOX18 protein compromises the expression and activation of the VCAM-1 promoter in all cell lines tested.** *A*, COS-1 cells were co-transfected with the empty luciferase vector pGL2Basic or the VCAM-1 promoter cloned into pGL2-Basic (pGL2-Basic-VC1889) and the empty vector pSG5 or pSG5 constructs driving the expression of wild type Sox18 or *Ra*, *RaJ*, *Ragl*, or *RaOP*. The results are expressed as the mean ± S.D. of 2–3 independent quadruplicates.

Transfection analysis in fibroblasts using the full-length VCAM-1 promoter demonstrate the inability of the four alleles of *ragged*, *Ra*, *RaJ*, *Ragl*, and *RaOP*, to *trans*-activate the VCAM-1 promoter as compared with wild type *Sox18* (Fig. 7*A*, *lanes 12–16*). Furthermore, co-transfection of the VCAM reporter construct with equal amounts of wild type Sox18 and any of the four *ragged* mutants leads to complete repression of VCAM-1 *trans*-activation, suggesting that these mutants act in a dominant-negative fashion. This result is also observed in endothelial and skeletal muscle cells (data not shown).

VCAM-1 Expression in Lung, Skeletal Muscle, and Skin Is Significantly Reduced in the ragged Mouse—The phenotype of *ragged* mice, abnormal lymphatic system and hair follicles, is similar to that of humans with the disorder referred to as hypotrichosis-lymphedema-telangiectasia (20). Our *in vitro* and transfection analysis established Sox18 as crucial for the *trans*-activation of VCAM-1 expression in cells and show that *ragged* proteins target and prevent VCAM-1 activation by Sox18.

Of the four alleles of *ragged*, *RaOP* is the most deleterious to the survival of the offspring. The *RaOP* heterozygotes resemble the homozygotes of the other three allelic forms and generally display a hairless and edematous phenotype (52, 53). Therefore, we utilized the *RaOP* mouse model to investigate the *in vivo* effect of this mutant form of Sox18 on the expression levels of VCAM-1. By using real time RT-PCR with RNA from lung, skin, and skeletal muscle, organs which exhibit high expression of Sox18 and VCAM-1 (10, 26–30, 48), we demonstrated aberrant VCAM-1 expression in this mutant. Fig. 8 vividly demonstrates the effect that the *RaOP* mutation exerts on the expression of VCAM-1 in these tissues. In the wild type lung, VCAM-1 is abundantly expressed, in contrast to the mutant animal, which expresses 5-fold less. Moreover, the *RaOP* mutation extensively abrogates expression of VCAM-1 in skeletal muscle and skin, with levels of repression up to almost 40-fold (the data and normalization are described in Table I). Moreover, the specificity of aberrant VCAM-1 mRNA expression in the *ragged* mouse is highlighted by our observation that ICAM-1 and −2, JCAM, NCAM-1, and PECAM-1 mRNA expressions are unaffected in *Ra* animals (data not shown). Thus, the *in vivo* evidence is in strong concordance with the *in vitro* and cell culture analyses. In conclusion, we provide strong evidence that the *VCAM-1* gene is a primary target of SOX18 and that its expression is significantly compromised by all *ragged* Sox18 mutant proteins.

DISCUSSION

We present evidence that Sox18 regulates the expression of the important VCAM-1 that parallels the pattern of expression of Sox18. VCAM-1 is involved in the inflammatory response, and its aberrant regulation and/or expression has been implicated in edema and atherogenesis.

This validated our Sox18 target gene rationale based on overlapping spatio-temporal expression profiles. For example, the *Sox18* gene is expressed in the vasculature, hair follicle, allantois, and the yolk-sac blood islands during embryogenesis (10) with wide ranging expression in the adult, notably in the lung and hind limb skeletal muscle (45). This rationale was further developed by an examination of the *ragged* mouse abnormalities. The four allelic variants of the *Ra* phenotype are characterized by hypotrichosis (54), chylous ascites, and edema from mesenteric lymphatic dysfunction (18, 19), consistent with aberrant VCAM-1 expression. Moreover, VCAM-1 is an important counter receptor or ligand for α_4 integrins (31) and is expressed in the developing allantois, the mesoderm of the yolk sac, and the hematopoiesis sites in the yolk sac (32, 55), similar to Sox18. Furthermore, VCAM-1 is expressed in multiple organs (56) and has an important role in skeletal muscle (28, 29) and blood vascular and lymphatic endothelia (46, 57–59).

We observed that the murine VCAM-1 promoter demonstrated cell-specific transcription in a manner similar to that reported previously (43, 44). We identified three potential Sox binding sites in the VCAM-1 promoter and utilized EMSA assays to show that Sox18 bound efficiently and in a sequence-specific manner to all three potential Sox binding sites. However, a series of 5′ unidirectional deletions and mutagenesis demonstrated that the SoxB motif is necessary and critical for SOX18-dependent *trans*-activation and basal expression of the VCAM-1 promoter.

The effect of mutation at the SoxB site at nucleotide position −715 bp on basal activity in the cell types tested was an unexpected aspect of this investigation, as it has been previously reported that expression of the human VCAM-1 promoter is dependent on the IRF-2 element in muscle (44). This element is positioned between the TATA box at −32 bp and the translation start site in the human promoter (42). Furthermore, the IRF-2 site is conserved in the murine promoter in the same region.[2] Our study suggests that, in the context of the first 1889 bp of murine VCAM-1 promoter, the SoxB binding site at −715 bp is necessary and sufficient for the basal and Sox18-activated expression of VCAM-1 in fibroblasts, endothelial, and skeletal muscle cells.

In the context of understanding the Ra phenotype, we observed all *ragged* allelic forms of mutant SOX18 (*Ra, RaJ*, *Ragl*, and *RaOP*) failed to activate the VCAM-1 promoter. Furthermore, the *ragged* forms of SOX18 have the ability to ablate the *trans*-activation of VCAM-1 expression in the presence of native SOX18. These results suggest that *ragged* SOX18 proteins act in a dominant negative fashion, *i.e.* they are able to interfere with the role of the wild type protein. A similar situation has been reported for the Sox10 mutation in the human disease called the Waardenburg syndrome (60, 61), which results in the expression of a protein that is prematurely truncated shortly after the HMG box. This mutant has been shown to act in a dominant negative fashion by virtue of its

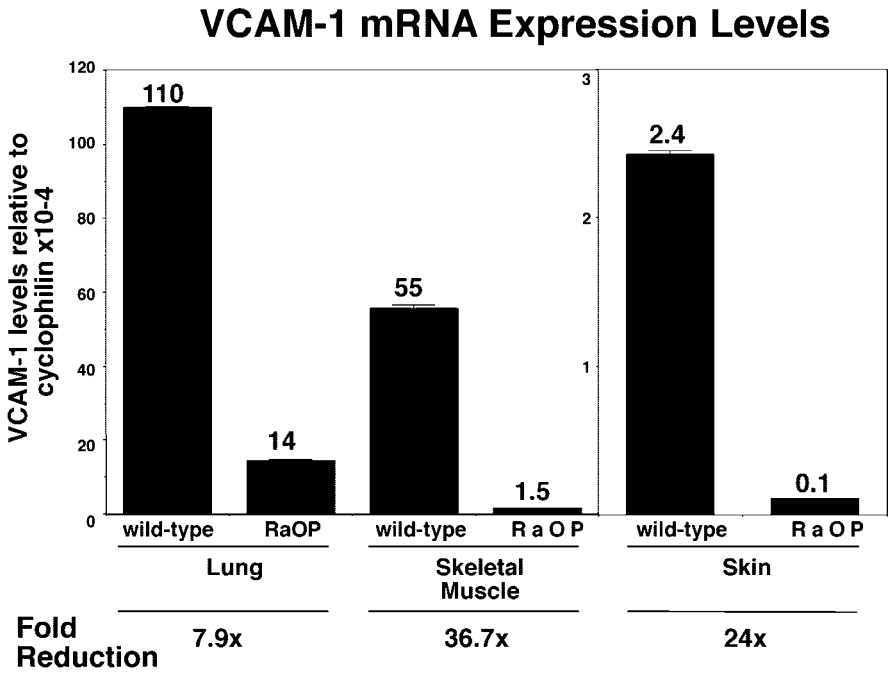

FIG. 8. **Real time quantitative RT-PCR comparison of VCAM-1 expression in wild type and *ragged* adult male sibling pairs.** The high expression tissues, lung, skin, and hind limb muscle were chosen for investigation of VCAM-1 expression levels. The levels of VCAM-1 mRNA were normalized and expressed relative to cyclophilin and calculated as displayed in Table I.

TABLE I
Raw real time data

This table demonstrates the calculation of relative levels of VCAM-1 from the raw real time RT-PCR data, carried out according to manufacturer's instructions.

Sample[a]	Ct[b]	ΔCt[c]	Rel. expr.[d] ($\times 10^{-4}$)
Wt lung cyclo	15.8		
RaOP lung cyclo	15.9		
Wt sk. mu. cyclo	17.4		
RaOP sk. mu. cyclo	18.3		
Wt Skin cyclo	16.9		
RaOP skin cyclo	17.8		
Wt lung VCAM	22.3	6.5	109.9 ± 0.3
RaOP lung VCAM	25.3	9.4	14.4 ± 0.36
Wt sk. mu. VCAM	24.8	7.5	55.4 ± 1.2
RaOP sk. mu. VCAM	31.1	12.7	1.5 ± 0.045
Wt skin VCAM	28.9	12	2.4 ± 0.023
RaOP skin VCAM	34.3	16.5	0.1 ± 0.00092

[a] Wt, wild type; cyclo, cyclophilin; sk. mu., skeletal muscle.
[b] Ct, crossing threshold.
[c] ΔCt, control crossing threshold − sample crossing threshold.
[d] Rel. Expr., relative expression ($2^{(\Delta Ct)}$).

ability to bind to but not activate the *Mitf* promoter (62), resulting in reduced levels of endogenous MITF protein. This causes the hypopigmentation and deafness associated with the Waardenburg syndrome. In a similar fashion to the SOX10 mutant, the ragged proteins bind to the DNA but do not *trans*-activate the expression of the *VCAM-1* promoter. In addition to competing for binding sites, the ragged proteins may act through a slower rate of turnover as compared with the wild type, or they may sequester co-factors that are in limited supply. All of these modes of action would render the wild type protein unable to activate the *VCAM-1* promoter and would be interpreted as a dominant negative effect.

It is hypothetically possible that expression of the ragged proteins may compromise the activity of other SOX proteins. Wegner (11) has stated that many SOX proteins have overlapping expression patterns and, in a few cases, it has been reported that multiple Sox factors can control the expression of the same genes in the same tissues. Thus, there is the distinct possibility that multiple SOX proteins can modulate the activity of VCAM-1 in endothelia and elsewhere where *ragged* is expressed. This leads us to the hypothesis that *ragged* may interfere with the ability of other SOX proteins to modulate the activity of VCAM-1, and this hypothesis is currently under investigation.

In choosing to study VCAM-1, we were interested in elucidating aspects of the molecular mechanisms mediating edema in *ragged* mice and in humans with HLT syndrome. It has been reported that an overexpression of VCAM-1 leads to inflammation and an edema that is characterized by an immune infiltrate (63–65). The *ragged* animal has been characterized with an edematous phenotype, and, yet, we have demonstrated here a marked reduction in the level of VCAM-1 expression. This suggests that the edema present in the *ragged* animal may not be linked to the expression of VCAM-1. If this were indeed the case, then it could be expected that the fluid retained in the interstitial spaces of the *ragged* animal would not consist of immune infiltrate. In agreement with this hypothesis, Wallace (19) has reported that the composition of the chyle present in some of the *ragged* homozygous neonates contained very few monocytes and macrophages. This conclusion is also supported by the fact that no edema was reported in mice targeted for VCAM-1 (35). These results suggest that misregulation of VCAM-1 is not the source of the edema and that, potentially, the lack of immune cells in the chyle of the *RaOP* animals may be due to the repression of VCAM-1 on the endothelia of the lymphatics. However, we cannot rule out that edema is the result of aberrant VCAM-1 expression, including overexpression or repression/ablation.

In conclusion, we show that SOX18 is essential for the expression of the cell adhesion molecule VCAM-1 (and not other adhesion molecules) in cell culture and animal models. The uncontrolled overexpression of VCAM-1 is associated with many chronic immune diseases such as atherosclerosis, arthritis, and asthma (66–68). In fact, much literature now reports the use of drugs that modulate VCAM-1 expression to successfully reduce immune infiltrate and the consequent edema in many diseases (69–71). We have shown that the −715 bp SoxB binding site regulates the VCAM-1 promoter activity and suggest that this site and Sox18 are therapeutic targets for the modulation of VCAM-1 expression and the control of inflammation.

Acknowledgments—We thank Jenny Rowland for the gift of mouse genomic DNA and Dr. Dagmar Wilhelm for manuscript preparation and instructive discussions about the investigation herein presented.

REFERENCES

1. Risau, W. (1997) *Nature* **386,** 671–674
2. Coffin, J. D., and Poole, T. J. (1991) *Anat. Rec.* **231,** 383–395
3. Deleted in proof
4. Deleted in proof
5. Deleted in proof
6. Deleted in proof
7. Deleted in proof
8. Deleted in proof
9. Deleted in proof
10. Pennisi, D., Gardner, J., Chambers, D., Hosking, B., Peters, J., Muscat, G., Abbott, C., and Koopman, P. (2000) *Nat. Genet.* **24,** 434–437
11. Wegner, M. (1999) *Nucleic Acids Res.* **27,** 1409–1420
12. Hosking, B. M., Muscat, G. E., Koopman, P. A., Dowhan, D. H., and Dunn, T. L. (1995) *Nucleic Acids Res.* **23,** 2626–2628
13. Koopman, P., Gubbay, J., Vivian, N., Goodfellow, P., and Lovell-Badge, R. (1991) *Nature* **351,** 117–121
14. Foster, J. W., and Graves, J. A. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1927–1931
15. James, K., Hosking, B., Gardner, J., Muscat, G. E., and Koopman, P. (2003) *Genesis* **36,** 1–6
16. Slee, J. (1957) *J. Genet.* **55,** 570–584
17. Slee, J. (1957) *J. Genet.* **55,** 100–121
18. Herbertson, B. M., and Wallace, M. E. (1964) *J. Med. Genet.* **1,** 10–23
19. Wallace, M. E. (1979) *Heredity* **43,** 9–18
20. Irrthum, A., Devriendt, K., Chitayat, D., Matthijs, G., Glade, C., Steijlen, P. M., Fryns, J. P., Van Steensel, M. A., and Vikkula, M. (2003) *Am. J. Hum. Genet.* **72,** 1470–1478
21. Steinman, L., and Zamvil, S. (2003) *Nat. Rev. Immunol.* **3,** 483–492
22. Smolen, J. S., and Steiner, G. (2003) *Nat. Rev. Drug Discov.* **2,** 473–488
23. Schieffer, B., and Drexler, H. (2003) *Am. J. Cardiol.* **91,** 12H–18H
24. DeGraba, T. J. (2003) *Adv. Neurol.* **92,** 29–42
25. Krieglstein, C. F., and Granger, D. N. (2001) *Am. J. Hypertens.* **14,** 44S–54S
26. Groves, R. W., Ross, E. L., Barker, J. N., and MacDonald, D. M. (1993) *J. Am. Acad. Dermatol.* **29,** 67–72
27. Davies, D., Larbi, K., Allen, A., Sanz, M., Weg, V. B., Haskard, D. O., Lobb, R. R., and Nourshargh, S. (1999) *Immunology* **97,** 150–158
28. Rosen, G. D., Sanes, J. R., LaChance, R., Cunningham, J. M., Roman, J., and Dean, D. C. (1992) *Cell* **69,** 1107–1119
29. Stepp, M. A., Urry, L. A., and Hynes, R. O. (1994) *Cell Adhes. Commun.* **2,** 359–375
30. Beck-Schimmer, B., Schimmer, R. C., Madjdpour, C., Bonvini, J. M., Pasch, T., and Ward, P. A. (2001) *Am. J. Respir. Cell Mol. Biol.* **25,** 780–787
31. Elices, M. J., Osborn, L., Takada, Y., Crouse, C., Luhowskyj, S., Hemler, M. E., and Lobb, R. R. (1990) *Cell* **60,** 577–584
32. Chan, B. M., Elices, M. J., Murphy, E., and Hemler, M. E. (1992) *J. Biol. Chem.* **267,** 8366–8370
33. Postigo, A. A., Sanchez-Mateos, P., Lazarovits, A. I., Sanchez-Madrid, F., and de Landazuri, M. O. (1993) *J. Immunol.* **151,** 2471–2483
34. Gurtner, G. C., Davis, V., Li, H., McCoy, M. J., Sharpe, A., and Cybulsky, M. I. (1995) *Genes Dev.* **9,** 1–14
35. Cybulsky, M. I., Iiyama, K., Li, H., Zhu, S., Chen, M., Iiyama, M., Davis, V., Gutierrez-Ramos, J. C., Connelly, P. W., and Milstone, D. S. (2001) *J. Clin. Investig.* **107,** 1255–1262
36. Barks, J. L., McQuillan, J. J., and Iademarco, M. F. (1997) *J. Immunol.* **159,** 4532–4538
37. Briscoe, D. M., Cotran, R. S., and Pober, J. S. (1992) *J. Immunol.* **149,** 2954–2960
38. Park, S. K., Yang, W. S., Lee, S. K., Ahn, H., Park, J. S., Hwang, O., and Lee, J. D. (2000) *Nephrol. Dial. Transplant.* **15,** 596–604
39. Henninger, D. D., Panes, J., Eppihimer, M., Russell, J., Gerritsen, M., Anderson, D. C., and Granger, D. N. (1997) *J. Immunol.* **158,** 1825–1832
40. Kim, I., Moon, S. O., Kim, S. H., Kim, H. J., Koh, Y. S., and Koh, G. Y. (2001) *J. Biol. Chem.* **276,** 7614–7620
41. Kim, I., Moon, S. O., Park, S. K., Chae, S. W., and Koh, G. Y. (2001) *Circ. Res.* **89,** 477–479
42. Jesse, T. L., LaChance, R., Iademarco, M. F., and Dean, D. C. (1998) *J. Cell Biol.* **140,** 1265–1276
43. Iademarco, M. F., McQuillan, J. J., Rosen, G. D., and Dean, D. C. (1992) *J. Biol. Chem.* **267,** 16323–16329
44. Iademarco, M. F., McQuillan, J. J., and Dean, D. C. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3943–3947
45. Hosking, B. M., Wyeth, J. R., Pennisi, D. J., Wang, S. C., Koopman, P., and Muscat, G. E. (2001) *Gene* **262,** 239–247
46. Korenaga, R., Ando, J., Kosaki, K., Isshiki, M., Takada, Y., and Kamiya, A. (1997) *Am. J. Physiol.* **273,** C1506–C1515
47. Chen, S. L., Dowhan, D. H., Hosking, B. M., and Muscat, G. E. (2000) *Genes Dev.* **14,** 1209–1228
48. Hosking, B. M., Wang, S. C., Chen, S. L., Penning, S., Koopman, P., and Muscat, G. E. (2001) *Biochem. Biophys. Res. Commun.* **287,** 493–500
49. Klimiuk, P. A., Sierakowski, S., Latosiewicz, R., Cylwik, J. P., Cylwik, B., Skowronski, J., and Chwiecko, J. (2002) *Ann. Rheum. Dis.* **61,** 804–809
50. Quandt, K., Frech, K., Karas, H., Wingender, E., and Werner, T. (1995) *Nucleic Acids Res.* **23,** 4878–4884
51. Kanai, Y., Kanai-Azuma, M., Noce, T., Saido, T. C., Shiroishi, T., Hayashi, Y., and Yazaki, K. (1996) *J. Cell Biol.* **133,** 667–681
52. Green, E. L., and S. J., M. (1961) *J. Hered.* **52,** 223–227
53. Mann, S. J. (1963) *Genet. Res.* **4,** 1–11
54. Carter, T. C., and Phillips, J. S. (1953) *J. Hered.* **45,** 151–154
55. Kwee, L., Baldwin, H. S., Shen, H. M., Stewart, C. L., Buck, C., Buck, C. A., and Labow, M. A. (1995) *Development* **121,** 489–503
56. Sheppard, A. M., Onken, M. D., Rosen, G. D., Noakes, P. G., and Dean, D. C. (1994) *Cell Adhes. Commun.* **2,** 27–43
57. Sawa, Y., Shibata, K., Braithwaite, M. W., Suzuki, M., and Yoshida, S. (1999) *Microvasc. Res.* **57,** 100–106
58. Young, A. J., Seabrook, T. J., Marston, W. L., Dudler, L., and Hay, J. B. (2000) *Eur. J. Immunol.* **30,** 327–334
59. Dyer, C. M., and Lew, A. M. (2003) *Vaccine* **21,** 2124–2130
60. Kuhlbrodt, K., Schmidt, C., Sock, E., Pingault, V., Bondurand, N., Goossens, M., and Wegner, M. (1998) *J. Biol. Chem.* **273,** 23033–23038
61. Pingault, V., Bondurand, N., Kuhlbrodt, K., Goerich, D. E., Prehu, M. O., Puliti, A., Herbarth, B., Hermans-Borgmeyer, I., Legius, E., Matthijs, G., Amiel, J., Lyonnet, S., Ceccherini, I., Romeo, G., Smith, J. C., Read, A. P., Wegner, M., and Goossens, M. (1998) *Nat. Genet.* **18,** 171–173
62. Lee, M., Goodall, J., Verastegui, C., Ballotti, R., and Goding, C. R. (2000) *J. Biol. Chem.* **275,** 37978–37983
63. Abe, Y., Sugisaki, K., and Dannenberg, A. M., Jr. (1996) *J. Leukocyte Biol.* **60,** 692–703
64. Harrison, A. A., Stocker, C. J., Chapman, P. T., Tsang, Y. T., Huehns, T. Y., Gundel, R. H., Peters, A. M., Davies, K. A., George, A. J., Robinson, M. K., and Haskard, D. O. (1997) *J. Immunol.* **159,** 4546–4554
65. Willuweit, A., Sass, G., Schoneberg, A., Eisel, U., Tiegs, G., and Clauss, M. (2001) *J. Immunol.* **167,** 3944–3952
66. Cybulsky, M. I., and Gimbrone, M. A., Jr. (1991) *Science* **251,** 788–791
67. Morales-Ducret, J., Wayner, E., Elices, M. J., Alvaro-Gracia, J. M., Zvaifler, N. J., and Firestein, G. S. (1992) *J. Immunol.* **149,** 1424–1431
68. Montefort, S., Holgate, S. T., and Howarth, P. H. (1993) *Eur. Respir. J.* **6,** 1044–1054
69. Yamazaki, R., Hatano, H., Aiyama, R., Matsuzaki, T., Hashimoto, S., and Yokokura, T. (2000) *Eur. J. Pharmacol.* **404,** 375–385
70. Feeley, B. T., Park, A. K., Hoyt, E. G., and Robbins, R. C. (1999) *J. Heart Lung Transplant.* **18,** 1088–1095
71. Pierce, J. W., Schoenleber, R., Jesmok, G., Best, J., Moore, S. A., Collins, T., and Gerritsen, M. E. (1997) *J. Biol. Chem.* **272,** 21096–21103

The *VCAM-1* Gene That Encodes the Vascular Cell Adhesion Molecule Is a Target of the Sry-related High Mobility Group Box Gene, *Sox18*

Brett M. Hosking, S.-C. Mary Wang, Meredith Downes, Peter Koopman and George E. O. Muscat

J. Biol. Chem. 2004, 279:5314-5322.
doi: 10.1074/jbc.M308512200 originally published online November 21, 2003
