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Down syndrome candidate region 1-like 1 (DSCR1-L1) mimics the inhibitory effects of DSCR1 on calcineurin signaling in endothelial cells and inhibits angiogenesis*

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Abstract

In endothelial cells, binding of vascular endothelial growth factor (VEGF) to VEGF receptor 2 (VEGFR-2) leads to the activation of the serine/threonine phosphatase calcineurin, dephosphorylation of the NF-AT transcription factors, translocation of NF-AT to the nucleus, and expression of angiogenesis-related genes such as Cox-2. Down syndrome candidate region 1 (DSCR1) is transactivated by NF-AT nuclear translocation, and in turn inhibits calcineurin activity, forming a negative feedback loop. While DSCR1 has a clearly defined role as an endogenous inhibitor of VEGF-calcineurin mediated angiogenesis in endothelial cells, the function of the DSCR1 family member, DSCR1-like1 (DSCR1-L1) has not yet been investigated in endothelial cells. Here we show that a panel of proangiogenic factors including VEGF, basic fibroblast growth factor (bFGF), angiopoietin 1 (Ang1), hepatocyte growth factor (HGF) as well as triiodo-L-thyronine (T3) does not induce DSCR1-L1 upregulation in endothelial cells while VEGF potently upregulates DSCR1. To investigate the effects of DSCR1-L1 on endothelial cell function, we cloned the gene into a lentiviral vector and over-expressed DSCR1-L1 in human umbilical vein endothelial cells (HUVEC). Constitutive DSCR1-L1 over-expression prevented the nuclear translocation of NF-ATc1 in response to VEGF, underscoring its role as a calcineurin inhibitor. Additionally, DSCR1-L1-transduced cells inhibited VEGF-induced endothelial cell migration, proliferation, and tube formation by 36%, 77%, and 39%, respectively, compared to cells infected with control virus. Over-expression of DSCR1-L1 in the transformed endothelial cell line SVR also resulted in decreased proliferation. Our findings demonstrate that DSCR1-L1 is constitutively expressed in endothelial cells and acts similar to DSCR1 in inhibiting calcineurin activity and restraining VEGF-mediated angiogenesis.

INTRODUCTION

Calcineurin (PP2B) is a serine/threonine phosphatase that is activated upon elevations in intracellular calcium (1). Activated calcineurin triggers the nuclear import of nuclear factor of activated T cells (NF-AT) transcription factors and drives expression of cell-specific genes,

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most notably in the cardiovascular, nervous, and immune systems (2;3). The significance of calcineurin function in the immune system is highlighted by the effects of the immunosuppressive drugs cyclosporine A and FK506, which inhibit calcineurin activity causing potent immunosuppression (4). A family of three endogenous calcineurin regulators has been described and was originally named the Down syndrome candidate region gene and homologs (DSCR1, DSCR1-L1, and DSCR1-L2) due to its location on the minimal supernumerary fragment of chromosome 21 in individuals with Down syndrome (5). Other names for this family include the calcipressins (Csp1, Csp2, and Csp3) (6) and the modulatory calcineurin interacting proteins (MCIP1, 2, and 3) (7). We previously demonstrated that DSCR1 inhibits calcineurin activity in the immune system, with loss of DSCR1 causing calcineurin overactivity and ultimately leading to Fas ligand mediated apoptosis of T cells (8).

The calcium-calcineurin- NF-AT pathway has been more recently demonstrated to be an important mediator of vascular endothelial growth factor (VEGF) signaling in endothelial cells. VEGF is critical for endothelial cell function (9). Binding of VEGF to its primary receptor, VEGFR-2 (KDR/Flk-1), stimulates a variety of signaling factors including phospholipase Cγ, resulting in a rapid increase of intracellular calcium (10). Activated calcineurin dephosphorylates and induces the nuclear import of NF-ATc1 and NF-ATc2 in endothelial cells, resulting in the expression of angiogenesis-related genes including Cox-2 and E-selectin (11–13). Studies have demonstrated that DSCR1 is upregulated in response to VEGF in a calcineurin-dependent manner (14–17), and that over-expression of DSCR1 in endothelial cells inhibits NF-AT translocation, NF-AT-mediated gene expression, and endothelial cell function (14–17).

DSCR1-L1, also called ZAKI-4, calcipressin 2 (Csp2), and modulatory calcineurin interacting protein 2 (MCIP2), was originally cloned from human skin fibroblasts treated with triiodo-L-thyronine (T_3) (18). The DSCR1-L1 gene is located on chromosome 6 and contains a conserved sequence encoding a calcineurin-binding motif that is common to all three DSCR1 family members (7). DSCR1-L1 has two isoforms, α and β, with the α isoform expressed primarily in the brain and the β isoform expressed ubiquitously (19). Unlike DSCR1, DSCR1-L1 is not induced by a calcium-dependant mechanism. The addition of the calcium ionophore ionomycin has no effect DSCR1-L1 levels, while DSCR1 is strongly induced (20). T_3 treatment of fibroblasts results in significant α isoform upregulation but only minimal β isoform upregulation. Both α and β isoforms contain the conserved calcineurin binding motif and both isoforms block calcineurin activity when transfected into CHO cells or Jurkat cells (19).

Currently, T_3 is the only known inducer of DSCR1-L1. Given the role of DSCR1 in mediating VEGFR2-activated calcineurin signaling in endothelial cells, we sought to determine if VEGF or other angiogenic factors could upregulate DSCR1-L1 in endothelial cells and to determine if DSCR1-L1 could inhibit endothelial cell function similar to DSCR1. We found that DSCR1-L1 is constitutively expressed in endothelial cells and that levels were unaffected by treatment with any angiogenic factor tested. Further, we demonstrate that DSCR1-L1 is as effective as DSCR1 in blocking endothelial cell responses to VEGF.

MATERIAL AND METHODS

Cells

Human umbilical vein endothelial cells (HUVEC, Cambrex, East Rutherford, NJ) were maintained in EGM-2-MV (Cambrex) on 0.2% gelatin-coated plates, and used between passages 2 and 6. Sven 1 ras cells (SVR) were obtained from ATCC (Manassas, VA) and maintained in DMEM with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% l-

glutamine (complete media). 293FT cells were purchased from Invitrogen (Carlsbad, CA), and maintained in complete media.

Quantitative Real-Time PCR

HUVEC were plated at 4×10^4 cells/ml and starved overnight in Optimem with 1% FBS. Angiopoietin-1 (20 ng/ml), human recombinant basic fibroblast growth factor (10 ng/ml), human recombinant hepatocyte growth factor (10 ng/ml), 3,5,3′ triiodo-L-thyronine (T_3) (10 uM) (all from Sigma, St. Louis, MO) and human recombinant vascular endothelial growth factor (10 ng/ml) (National Cancer Institute Preclinical Repository, Rockville, MD) were applied to HUVEC for 1 hr. Stimulated cells were harvested in TRIzol (Invitrogen, Carlsbad, CA), and mRNA was chloroform extracted. cDNA was reverse transcribed using random hexamers from the SuperScript® First Strand Synthesis kit (Invitrogen, Carlsbad, CA). Reactions were run in LightCycler® capillaries (20ul) using LightCycler® FastStart DNA MasterPLUS Sybr Green I on the LightCycler Thermal Cycler 1.0 (Roche Applied Science, Indianapolis, IN). All primers were synthesized by Integrated DNA Technologies (Coralville, IA). DSCR1 primers were the following: 5′-tgcgaccccagtcataaacta-3′ and 5′-ccatttcctcttcttcctcctt-3′. DSCR1-L1 primers were the following: 5′-atgacctcctctatgctgtgg-3′, 5′-tttggatgatttttggctttg-3′. Primers were designed to yield 150–200 bp products. Results were normalized to 18S ribosomal RNA using QuantumRNA 18S Internal Standards (Ambion, Austin, TX) using a linear formula. All samples were run in duplicate.

Retroviral and lentiviral construction

The retroviral construct pMX.myc.DSCR1 was constructed as previously described (8). The retroviral construct pMX was used a control. Ecotropic retroviruses were produced by transfection of the PHOENIX ECO packaging line (provided by Dr. G.P. Nolan, Stanford University Medical Center) with 1 *u*g DNA using TRANS-IT 293 (Mirus, Madison, WI). Viral supernatants were collected on day 5 and 7 after transfection and passed through a 0.45 *u*M filter before storage at −80°C. Endothelial cells were infected with retrovirus in the presence of 8 *u*g/ml polybrene.

The lentiviral construct pLenti4/V5.GW.lacZ was purchased from Invitrogen (Carlbad, CA). pLenti4.V5.DSCR1-L1 was created using the ViraPower Lentiviral Expression System (Invitrogen). DSCR1-L1 cDNA with attB cloning sites on both ends was created by PCR amplification using the following primers:

5′-ggggacaagtttgtacaaaaaagcaggctcccctagcatggactgtgatgtttc-3′

5′-ggggaccactttgtacaagaaagctgggtagttggacacggagggtggc-3.

An intermediate plasmid vector was generated by inserting the PCR product into vector pDONR/Zeo using BP Clonase following manufacturer's instructions. Reaction products were transformed into DH5α competent *E. coli* and resistant colonies were selected. Correctly sequenced products in the pDONR/Zeo inserted into pLenti4/V5-DEST using LR Clonase to create pLenti4/V5.DSCR1-L1. Reaction products were transformed into DH5α competent *E. coli* and resistant colonies selected and expanded. Plasmid was isolated using either Qiagen plasmid mini or midi kits (Valencia, CA). Products were sequenced to ensure that mutations were not introduced. Lentivirus was generated by transfecting pLenti4/V5.DSCR1-L1 and pLenti4/V5.GW.lacZ along with ViraPower Packaging Mix into 293FT cells according to the manufacturer's instructions. Media was changed the following day, and supernatant containing active virus was collected 2–3 days after transfection. Media was spun at 3000 rpm for 15 minutes and supernatant was stored at −80°C. Endothelial cells were infected with lentivirus in the presence of 8 *u*g/ml polybrene.

Transgene expression was confirmed by infecting 293 HEK cells with pMX.myc.DSCR1, pMX, pLenti4/V5.GW.lacZ, pLenti4/V5.DSCR1 and pLenti4/V5.DSCR1-L1. Infected cells were lysed in 2X SDS buffer and run on a Tris-glycine gels. Protein was transferred to a nitrocellulose membrane and blotted with anti-myc mouse monoclonal antibody (1:1000; Abcam, Cambridge, MA) or anti-V5 mouse monoclonal antibody (1:200, Invitrogen, Carlsbad, CA) and 1:1000 goat anti-mouse HRP conjugated secondary antibody (1:1000 Zymed, San Francisco, CA). Proteins were detected using the Enhanced Chemiluminescence (ECL) kit (Amersham Biosciences, Piscataway, NJ).

Transduction of endothelial cells

HUVEC were infected with pMX.myc.DSCR1, pMX, pLenti4/V5.GW.lacZ, pLenti4/V5.DSCR1 or pLenti4/V5.DSCR1-L1 in 10 cm dishes with 1 ml of unconcentrated viral supernatant with 2 mls media and 8 *u*g/ml polybrene. For retroviral infections, HUVEC were infected three times at 48 hr intervals and the media was changed the day following infection. For lentiviral infections, only one round of infection was performed, and the media was changed the day following infection. HUVEC were used 48–72 hrs after infection.

Immunofluorescence

Cells were plated onto 12 mm gelatin-coated coverslips, and starved overnight in Optimem with 1% FBS. Cells were left unstimulated or stimulated with 10 ng/ml VEGF in Optimem and fixed with 3% paraformaldehyde in PBS with 0.1% Triton X (PBST) for 10 minutes at 30 minutes and 1 hour. Fixed coverslips were blocked in 3% milk in PBST for 30 minutes. The mouse monoclonal antibody 7A6 (1:5; Santa Cruz Biotechnology) was used for detection of NF-ATc1. Cy3 conjugated goat anti-mouse IgG (1:1000; Molecular Probes, Eugene, OR) was used as the secondary antibody. Cell nuclei were stained with Hoechst dye (1*u*g/ml; Sigma). Images were obtained on a Zeiss microscope and analyzed using AxioVision 4.0 software (Carl Zeiss Vision).

Proliferation assay

HUVEC infected with retrovirus or lentivirus were plated at 2×10^4on 0.2% gelatin-coated 96 well plates. Cells were starved in Optimem with 1% FBS overnight. Cells were stimulated with VEGF (10 ng/ml) for 48 hours, pulsed with [^{3}H]-thymidine for 24 h, and harvested for liquid scintillation counting (Wallac, Gaithersburg, MD). Samples were performed in triplicate. Alternatively, infected SVR cells were plated 1000 cells/well in 96 well plates and then placed in Optimem with1% FBS overnight. The next day, VEGF 10ng/ml was added to the media. Cells were incubated for 24 and 72 hr, and the number of cells was quantified using a colorimeric MTT assay as previously described (21). Data reflect the mean of six samples.

Migration assay

Optimem with 1% FBS and VEGF (10 ng/ml) was placed in the lower chamber of a modified Boyden chamber (Neuroprobe, Cabin John, MD) and separated by a filter with 8 uM pores (Osmonics/GE Water Technologies, Trevose, PA). 4×10^5 HUVEC infected with retrovirus or lentivirus in Optimem with 1% FBS were plated in the upper chamber and allowed to migrate for 6 hours at 37°C. The filters were stained with Diff-Quick solution (Baxter, Miami, FL) and the number of cells that migrated across the filter was counted in 6 high-power fields (100x) per insert.

Matrigel tube formation assay

HUVEC infected with retrovirus or lentivirus were incubated in Optimem with 1% FBS overnight. Ice-cold Matrigel (BD Biosciences) containing 10 ng/ml VEGF was diluted 1:1 with RPMI and added to 96 well dishes and allowed to gel for 1 hour at 37°C. 1.5×10^5 HUVEC

were plated onto the wells. After 12 hours wells were stained with Diff-Quick solution (Baxter, Miami, FL) photographed, and numbers of complete capillary tubes were counted by a blinded observer.

RESULTS

VEGF treatment of endothelial cells leads to the potent upregulation of DSCR1 (22). Using quantitative real-time PCR, we confirmed that VEGF treatment of human umbilical vein endothelial cells (HUVEC) led to significant upregulation of DSCR1 (Fig. 1A). To determine if VEGF or other proangiogenic factors led to DSCR1-L1 in endothelial cells, HUVEC were treated with physiological concentrations of a panel of pro-angiogenic factors including angiopoietin 1 (Ang1), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), VEGF, and as well as T_3. Levels of DSCR1-L1 as assessed by quantitative real-time PCR were not significantly increased by any treatment suggesting that DSCR1-L1 is constitutively expressed in HUVEC (Fig. 1B).

To assess the effects of DSCR1-L1 over-expression in endothelial cells, a lentiviral vector (pLenti4.V5.DSCR1-L1) was constructed containing the DSCR1-L1 α isoform cDNA and a V5 epitope tag (Fig. 2A). Production of protein was confirmed by infecting HUVEC with retrovirus (pMX, pMX.myc.DSCR1) or lentivirus (pLenti4/V5.GW.lacZ, pLenti4/V5.DSCR1-L1) and performing a Western blot with whole cell lysates for the myc or V5 tag (Fig. 2B). It has been previously shown that DSCR1 and DSCR1-L1 inhibit calcineurin activity, preventing the dephosphorylation of NF-AT transcription factors (16;19). To confirm that over-expression of DSCR1-L1 blocked calcineurin activation and NF-AT nuclear translocation in endothelial cells, we infected HUVEC cells with either pLenti4/V5.DSCR1-L1 or pLenti4/V5.GW.lacZ and stimulated cells with VEGF. Over-expression of DSCR1-L1 in HUVEC prevented the nuclear localization of NF-ATc1 while the majority of cells infected with control virus showed NF-ATc1 nuclear localization after one hour of VEGF treatment (Fig. 2C).

Prior studies have demonstrated that DSCR1 inhibits endothelial cell proliferation, migration, and tube formation *in vitro* (14–17). To confirm these findings and to compare the effects of DSCR1 to DSCR1-L1, HUVEC were infected with our retroviral construct containing the DSCR1 gene (pMX.myc.DSCR1). DSCR1 over-expression in HUVEC resulted in a 43% decrease in VEGF-induced proliferation (Fig. 3A). When HUVEC migration toward VEGF was examined in a modified Boyden chamber, DSCR1 over-expression inhibited migration by 57% (Fig. 3B). Finally when HUVEC capillary tube formation was assessed on Matrigel, DSCR1 decreased the number of tubes formed after 12 hrs by 53% (Fig. 3C).

To investigate DSCR1-L1's potential as an endogenous inhibitor of VEGF-induced angiogenesis, we performed the same endothelial cell assays on HUVEC infected with the DSCR1-L1 or lacZ lentiviruses. Proliferation of DSCR-L1 over-expressing cells in response to VEGF was compared to uninfected cells and cells infected with control lentivirus. DSCR1-L1-infected cells proliferated 35% less compared to uninfected cells and 36% less compared to lacZ-infected cells (Fig. 4A). Constitutive expression of DSCR1-L1 inhibited HUVEC migration toward VEGF by 74% and 77% compared to uninfected cells and lacZ infected cells, respectively (Fig. 4B). The same lentivirus-infected HUVEC cells were used in tube formation assays on Matrigel, and demonstrated inhibition of endothelial cell tube formation by 50% in DSCR1-L1 over-expressing endothelial cells as compared to uninfected cells (Fig. 4C). Lentiviral infection with the control lacZ virus mildly decreased HUVEC tube formation (18%), which likely represents a toxic effect from lentiviral infection.

The above studies suggest that VEGF activates calcineurin activity in endothelial cells and drives proliferation, migration, and tube formation, and that these downstream effects are

inhibited by the endogenous calcineurin inhibitors DSCR1 and DSCR1-L1. In a separate study, we found in the angiosarcoma cell line SVR (derived from transformed endothelial cells) that DSCR1 activity was suppressed. In SVR cells, DSCR1 is rapidly degraded in lysosomes leading to increased calcineurin activity (manuscript submitted for publication). To determine if DSCR1-L1 over-expression could suppress calcineurin activity and decrease SVR proliferation, SVR cells were infected with DSCR1-L1 or lacZ lentivirus and proliferation was measured. After 3 days, over-expression of DSCR1-L1 inhibited SVR proliferation by 26% (Fig. 5A).

DISCUSSION

This study also examines for the first time DSCR1-L1's role in endothelial cells and angiogenesis. In comparison to DSCR1 inhibition of angiogenesis *in vitro*, we demonstrate that over-expression of DSCR1-L1 is equally effective in suppressing the angiogenic behavior of endothelial cells *in vitro*. We examined the effect of both DSCR1 and DSCR1-L1 over-expression on proliferation, migration, and tube formation in endothelial cells and found that all three phenotypes were significantly inhibited. DSCR1-L1 also inhibits calcineurin activity in the angiosarcoma cell line SVR, resulting in decreased proliferation.

The calcineurin pathway is an important mediator of VEGF signaling in endothelial cells. Binding of VEGF to VEGFR-2 activates calcineurin and results in the upregulation of pro-angiogenic genes such as Cox-2 (Fig. 5B) (16). Calcineurin activation also leads to increased expression of its endogenous inhibitor DSCR1, which forms a negative feedback loop to regulate calcineurin activity. This study demonstrates that unlike DSCR1, DSCR1-L1 expression is stable in endothelial cells and not regulated by VEGF or other proangiogenic factors. Inhibition of the VEGFR-2/calcineurin/NF-AT signaling pathway by DSCR1 and DSCR-L1 prevents activation of the normal VEGF transcriptional program resulting in altered endothelial cell function as measured by *in vitro* assays. Over-expression of DSCR1-L1 in the transformed endothelial cell line SVR also decreased SVR proliferation.

This study identifies DSCR1-L1 as functionally important in endothelial cells. Our work examines for the first time the regulation of DSCR1-L1 in endothelial cells. T_3 is the only known factor to cause DSCR1-L1 induction in human skin fibroblasts, but T_3 has no effect on DSCR1-L1 in endothelial cells. HUVEC have been shown to express thyroid hormone receptor β1(TRβ1), which is the primary mediator of T_3 induced gene expression (23). TRβ1 acts as a mobile transcription factor via a mechanism known as genomic action. Upon T_3 ligation to TRβ1, T_3 translocates to the nucleus where it binds to a cis-acting response element located upstream of target genes and promotes transcription. However, T_3 induction of DSCR1-L1 is not mediated via genomic action even in human skin fibroblasts as there is no thyroid hormone response element upstream of the DSCR1-L1 gene (19). While the active elements downstream of TRβ1 are partially elucidated, the exact mechanism of T_3-mediated DSCR1-L1 induction is not clear. Previous investigation has shown that DSCR1-L1 expression is isoform and tissue specific (19). We found that DSCR1-L1 expression in HUVEC is unaffected by a panel of proangiogenic factors. Thus additional work is necessary to identify what factors, if any, induce DSCR1-L1 in endothelial or other cell types.

It has previously been shown that VEGF treatment of endothelial cells causes NF-AT transcription factors to translocate to the nucleus and drive expression of angiogenesis-related genes(16;24) and that DSCR1 is induced as well (14). Both NF-ATc1 and NF-ATc2 are expressed in HUVEC and translocate to the nuclear in a calcineurin-dependent manner. Similar to DSCR1, DSCR1-L1 over-expression inhibits calcineurin activity, preventing nuclear localization of NF-ATc1. Constitutive over-expression of DSCR1-L1 inhibited NF-ATc1 nuclear translocation after VEGF treatment. Similar results have been previously been

demonstrated for DSCR1, where constitutively expressed DSCR1 in endothelial cells blocks NF-AT translocation. This suggests that there is functional redundancy in the DSCR family with both DSCR1 and DSCR1-L1 inhibiting VEGF-induced NF-ATc1 translocation by blocking calcineurin activity.

In another study, we examined three different human angiosarcoma tumors and found aberrant DSCR1 expression implying that disruption of this pathway may be contributing to the tumorigenic phenotype (manuscript submitted for publication). VEGF-mediated DSCR1 upregulation after VEGF treatment was blocked in angiosarcoma cell lines despite calcineurin activation as evidenced by NF-ATc1 import and transactivation of Cox-2. The loss of DSCR1 regulation of this transduction pathway may be a unique event in the progression of endothelial cells to endothelial cell-derived tumors, or angiosarcomas. Examination of SVR and other angiosarcoma cell lines demonstrated that lysosomal degradation of DSCR1 may contribute to increased calcineurin activity in angiosarcomas. We confirm the role of calcineurin activity in the SVR angiosarcoma cell line by inhibiting calcineurin activity with DSCR1-L1 over-expression, which reduces SVR proliferation.

The calcineurin pathway is an important mediator of VEGF signaling in endothelial cells. Inhibition of this pathway by DSCR1 and DSCR1-L1 prevents activation of the normal VEGF transcriptional program resulting in altered endothelial cell function. Thus targeting calcineurin in endothelial cells using these endogenous inhibitors may be an effective strategy in treating cancers through inhibition of VEGF-induced angiogenesis.

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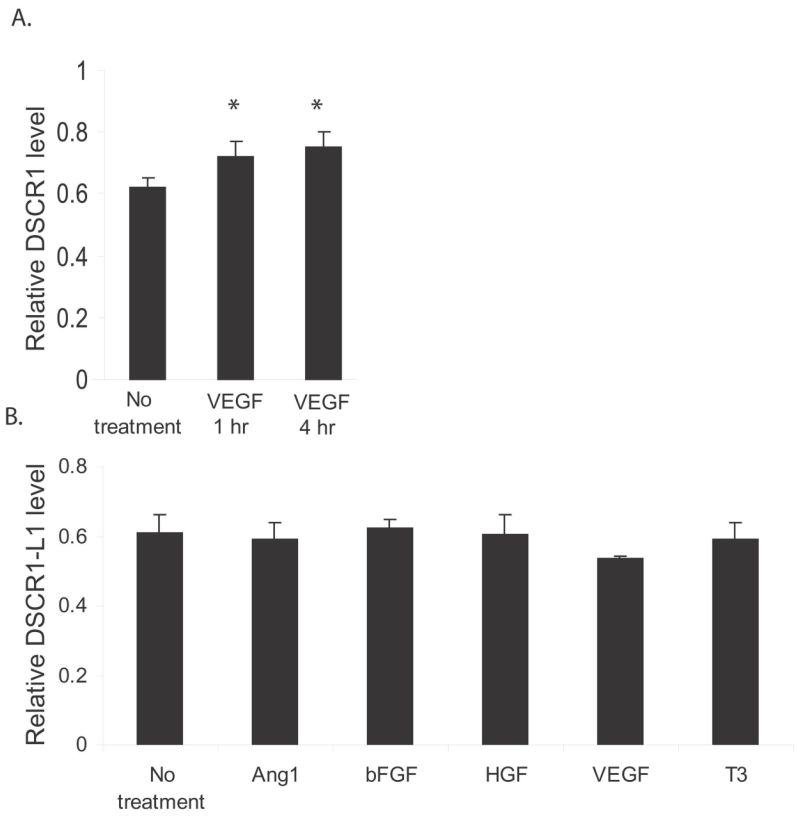

Figure 1.
Analysis of DSCR1 and DSCR1-L1 levels in response to pro-angiogenic factors. Relative mRNA levels of DSCR1 (A) and DSCR1-L1 (B) in response to pro-angiogenic stimuli. *p<0.05.

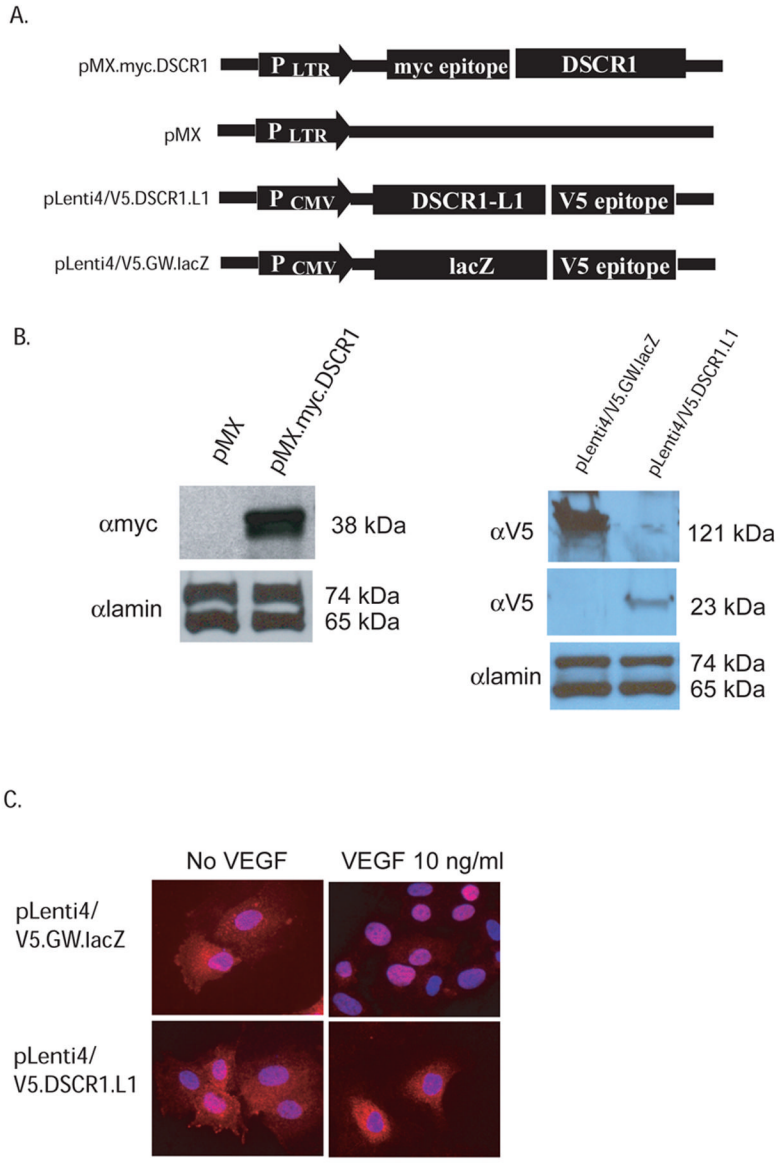

Figure 2.

DSCR1-L1 inhibition of calcineurin in endothelial cells. A. Schematic diagram of lentiviral constructs. B. Western blot analysis confirming DSCR1-L1 transgene expression. C. Immunofluorescence of NF-ATc1 subcellular localization in HUVEC infected with DSCR1-L1 lentivirus following VEGF treatment for 30 min or 1 hour.

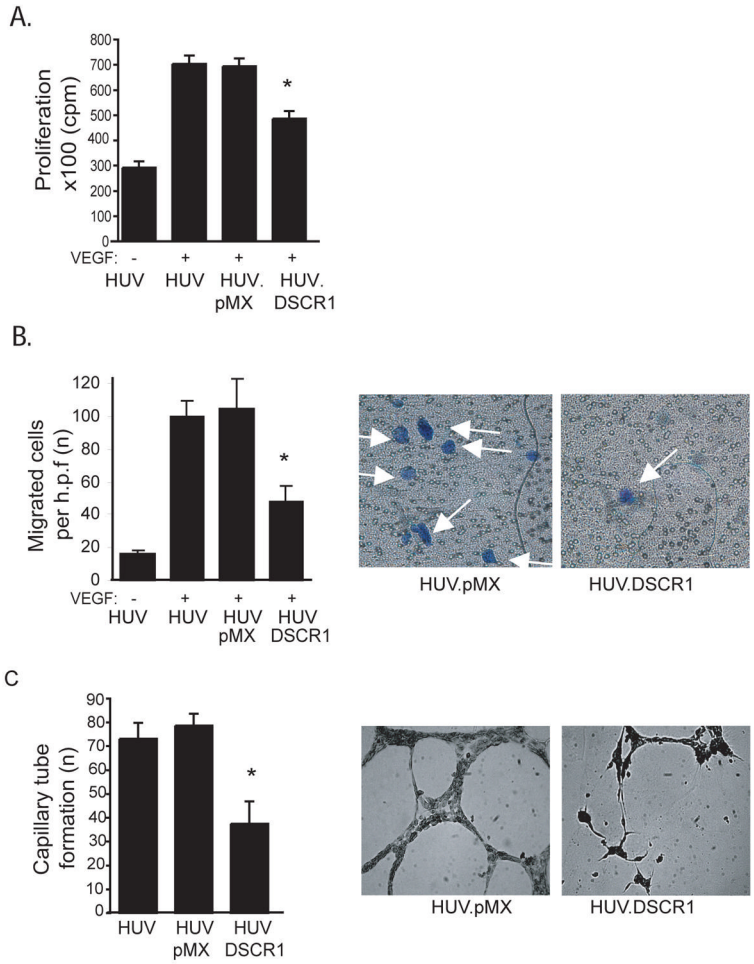

Figure 3.

Effect of DSCR1 over-expression on endothelial cell function. A. Proliferation of HUVEC (HUV), HUVEC infected with control virus pMC (HUV.pMX), and HUVEC infected with pMX.myc.DSCR1 (HUV.DSCR1) as measured by [^{3}H] thymidine uptake. Bars represent s.d. *$p < 0.01$ compared to HUV (+VEGF) and HUV.pMX. B. Migration of HUV.pMX or HUV.DSCR1 cells in response to VEGF after 6 hrs and representative photographs. Arrows point to migrated cells. Bars represent s.d. *$p < 0.01$ compared to HUV (+VEGF) and HUV.pMX. C. Capillary tube formation of HUV, HUV.pMX, and HUV.DSCR1 cells on Matrigel and representative photographs. Bars represent s.d. *$p < 0.01$ compared to HUV and HUV.pMX.

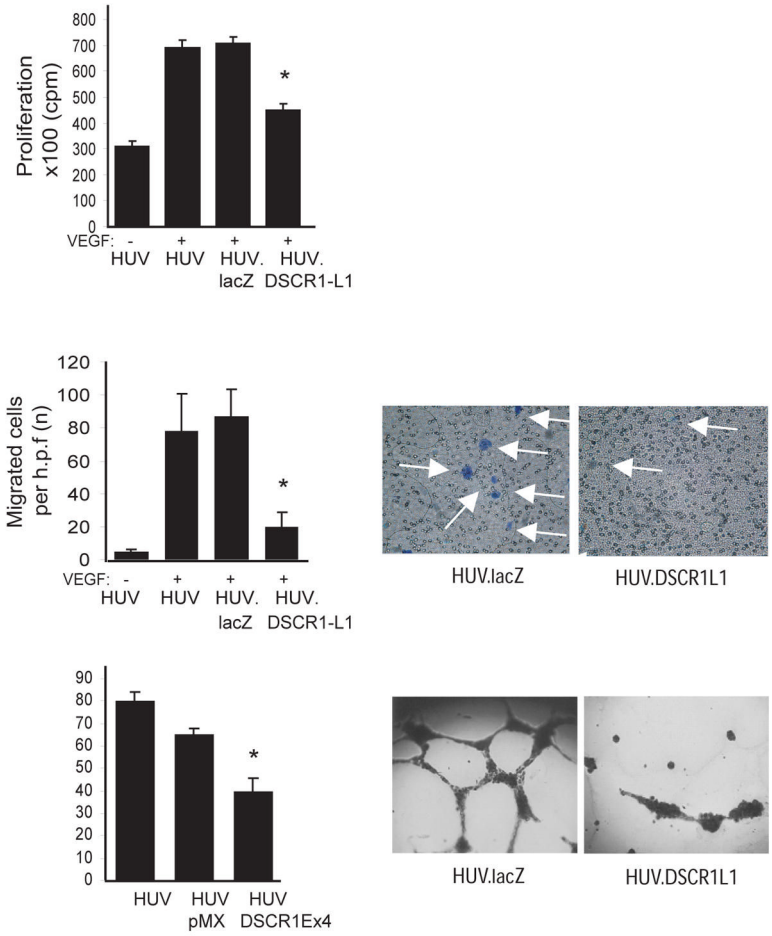

Figure 4.

Effect of DSCR1-L1 over-expression on endothelial cell function. A. Proliferation of HUVEC (HUV), HUVEC infected with control virus pLenti4/V5.DSCR1-L1 (HUV.lacZ), and HUVEC infected with pLenti4/V5.DSCR1-L1 (HUV.DSCR1-L1) as measured by [^{3}H] thymidine uptake. Bars represent s.d. *$p < 0.01$ compared to HUV (+VEGF) and HUV.lacZ. B. Migration of HUV.pMX or HUV.DSCR1 cells in response to VEGF after 6 hrs and representative photographs. Bars represent s.d. *$p < 0.01$ compared to HUV (+VEGF) and HUV.lacZ. Arrows point to migrated cells. C. Capillary tube formation of HUV, HUV.lacZ, and HUV.DSCR1-L1 cells on Matrigel and representative photographs. Bars represent s.d. *$p < 0.01$ compared to HUV and HUV.lacZ.

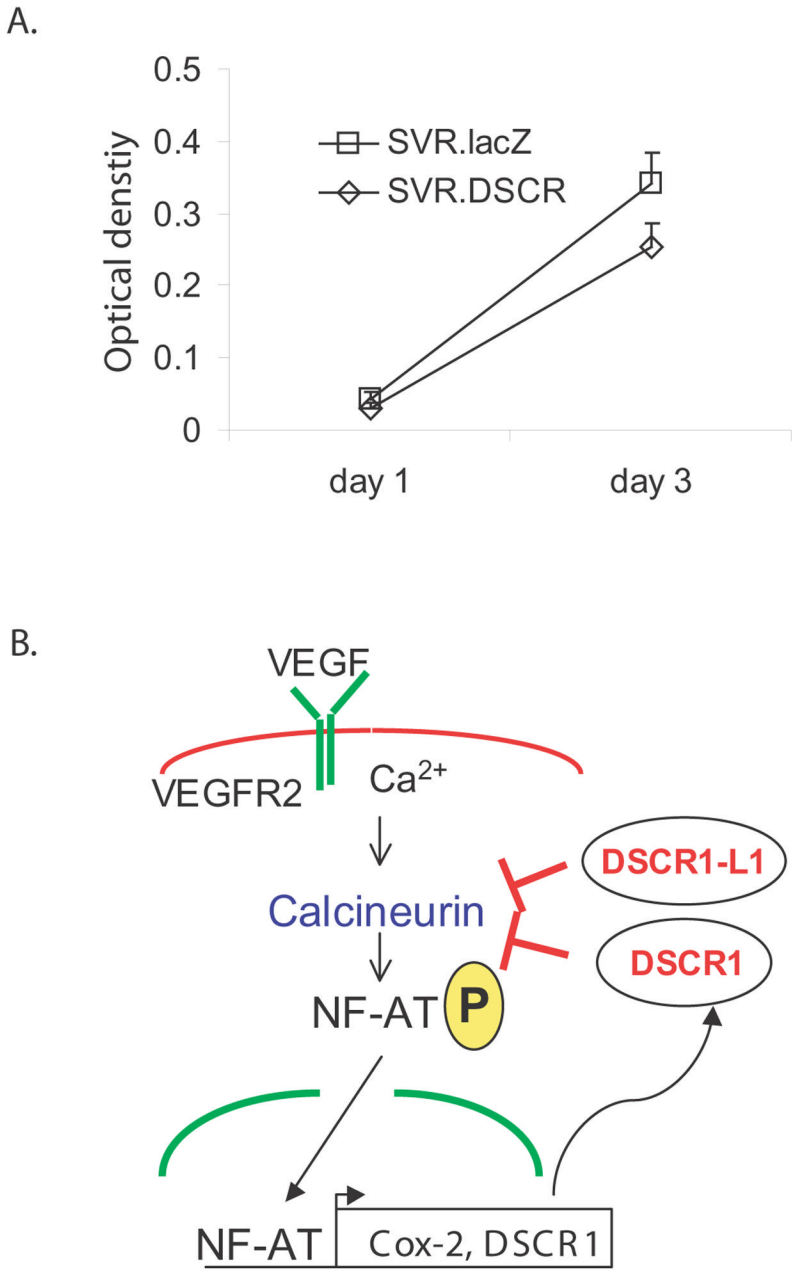

Figure 5.
Effect of DSCR1-L1 over-expression in SVR angiosarcoma cells on proliferation. A. Proliferation of SVR infected with pLenti4/V5.DSCR1-L1 (SVR.DSCR1) and SVR infected with control virus pLenti4/V5.DSCR1-L1 (SVR.lacZ) as measured by MTT colorimetric assay at 1 and 3 days. Bars represent s.d. B. Schematic diagram of the calcineurin/NF-AT pathway in endothelial cells.