

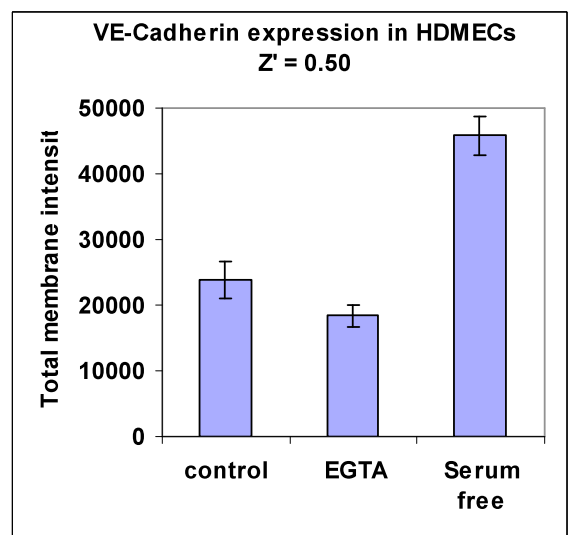
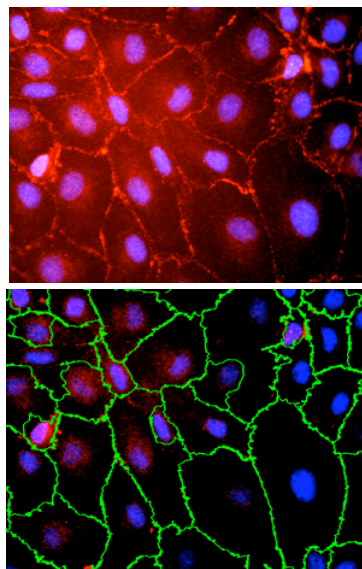
**Application note: Analysis of VE-cadherin expression in primary human dermal microvascular endothelial cells**

VE-cadherin (vascular endothelium cadherin, also known as Cadherin-5), is a member of cadherin superfamily, expressed by vascular endothelial cells (reviewed in 1-3). In these cells it is the major adherens junction protein, which, by modulating cell-cell adhesion, regulates angiogenesis, and vascular permeability. It is also required for differentiation into vasculature-like structures by endothelial cells in vitro (4, 5). Like other cadherins, VE-cadherin provides Ca<sup>2+</sup>-dependent homotypic adhesion between neighboring cells and serves as a link between plasma membrane and actin cytoskeleton. Additionally, unlike cadherins in epithelial cells, VE-cadherin also forms specialized adhesion structures linking to intermediate filaments (6, 7). Similar to other cadherins, VE-cadherin participates in signal transduction through Cdc42 (8), RhoA (9) and Rac (10) pathways, as well as via its dynamic associations with  $\beta$ -catenin and p120ctn. Endothelial monolayer permeability and the stability of the endothelial cell-cell adhesions can be modulated via endocytosis and degradation of VE-cadherin in a p120ctn-dependent manner (11, 12; reviewed in 3, 13). VE-cadherin has been shown to be the key factor in preventing proliferation of contact-inhibited endothelial cells (14). Conversely, inhibition of VE-cadherin correlates with increased cellular motility (15).

Since VE-cadherin is crucial for controlling the state of adherens junctions, which in turn regulate endothelial cell-cell adhesion, cell motility, morphogenesis and intracellular signaling pathways, this molecule has many clinical implications. For example, VE-cadherin expression is markedly reduced in vasculature-derived cancers, suggesting that this protein is important for tumor progression (16). On the other hand, highly aggressive melanomas (which are not endothelial in origin), acquire an ability to “mimic” blood vessel formation, and this process is accompanied by induction of VE-cadherin expression (17). Similarly, tumor-induced angiogenesis has been shown to involve upregulation of VE-cadherin in the neighboring vasculature (18, 19). Furthermore, VE-cadherin is actively involved during transendothelial migration of metastatic cancer cells (20, 21; reviewed in 22). Since blocking angiogenesis is a common strategy to inhibit tumor growth and extravasation, several approaches for inhibiting VE-cadherin function have been suggested as possible anti-tumor therapies (23-26). Finally, disruption of endothelial function during inflammation and pre-eclampsia has been shown to involve redistribution of VE-cadherin from the plasma membrane (27, 28).

Vala Sciences Inc has developed a reagent plus software kit to visualize and quantify VE-cadherin in cultured cells. The kit features staining reagents, optimized for staining cells cultured in the 96-well dish, and Vala’s CyteSeer® image analysis software platform. CyteSeer® is an affordable image cytometry program designed to analyze images acquired with virtually any digital microscopy workstation, and is

compatible with most computer operating systems. To demonstrate the capabilities of our kit, confluent primary human microvascular endothelial cells (HDMECs) were cultured under three condition: 1) in DMEM plus 10% FBS (control), 2) the same media containing 3 mM EGTA (which strongly reduces the calcium concentration of the culture media) for 3 hr, and 3) Overnight in serum-free media. The cells were then visualized for VE-cadherin using Vala’s reagent kit components, imaged using a Beckman IC100 robotic microscopy workstation (4 fields of view/well), and analyzed for VE-cadherin expression at the plasma membrane utilizing CyteSeer®. Incubation with EGTA led to an approximate 25% reduction in VE-cadherin association with the plasma membrane (Figure 1). Interestingly, overnight serum-withdrawal led to an approximate 100% increase in VE-cadherin visualized at the



**Figure 1.** Visualization of VE-cadherin in human microvascular endothelial cells. Upper left, Cells visualized for VE cadherin. Lower left, Mask generated by CyteSeer. Membrane associated VE-cadherin for cells cultured in EGTA or maintained in serum-free media. Data are the mean +/- SD for n=8 wells per condition. The wells were imaged (4 fields of view/well) with a Beckman IC100 high content microscopy workstation.

plasma membrane. For this experiment, the Z' value for the minimal and maximal responses (EGTA vs serum-starved cells) was 0.50, indicating that the assay is robust and suitable for quantitative image cytometry applications related to drug discovery or chemical genomics (29).

The goal of Vala Sciences Inc is to develop products enabling quantitative image cytometry for academic and pharmaceutical researchers. For further information please contact via our webpage, email, or phone.

**References**

1. Dejana, E. (1996). Endothelial adherens junctions: implications in the control of vascular permeability and angiogenesis. *J. Clin. Invest* 98, 1949-1953.
2. Dejana, E., Bazzoni, G., and Lampugnani, M.G. (1999). Vascular endothelial (VE)-cadherin: only an intercellular glue? *Exp. Cell Res.* 252, 13-19.
3. Vincent, P.A., Xiao, K., Buckley, K.M., and Kowalczyk, A.P. (2004). VE-cadherin: adhesion at arm's length. *Am. J. Physiol Cell Physiol* 286, C987-C997.

4. Feraud,O., Cao,Y., and Vittet,D. (2001). Embryonic stem cell-derived embryoid bodies development in collagen gels recapitulates sprouting angiogenesis. *Lab Invest* 81, 1669-1681.
5. Vittet,D., Buchou,T., Schweitzer,A., Dejana,E., and Huber,P. (1997). Targeted null-mutation in the vascular endothelial-cadherin gene impairs the organization of vascular-like structures in embryoid bodies. *Proc. Natl. Acad. Sci. U. S. A* 94, 6273-6278.
6. Kowalczyk,A.P., Navarro,P., Dejana,E., Bornslaeger,E.A., Green,K.J., Kopp,D.S., and Borgwardt,J.E. (1998). VE-cadherin and desmoplakin are assembled into dermal microvascular endothelial intercellular junctions: a pivotal role for plakoglobin in the recruitment of desmoplakin to intercellular junctions. *J. Cell Sci.* 111 ( Pt 20), 3045-3057.
7. Gallicano,G.I., Kouklis,P., Bauer,C., Yin,M., Vasioukhin,V., Degenstein,L., and Fuchs,E. (1998). Desmoplakin is required early in development for assembly of desmosomes and cytoskeletal linkage. *J. Cell Biol.* 143, 2009-2022.
8. Kouklis,P., Konstantoulaki,M., and Malik,A.B. (2003). VE-cadherin-induced Cdc42 signaling regulates formation of membrane protrusions in endothelial cells. *J. Biol. Chem.* 278, 16230-16236.
9. Nelson,C.M., Pirone,D.M., Tan,J.L., and Chen,C.S. (2004). Vascular endothelial-cadherin regulates cytoskeletal tension, cell spreading, and focal adhesions by stimulating RhoA. *Mol. Biol. Cell* 15, 2943-2953.
10. Lampugnani,M.G., Zanetti,A., Breviario,F., Balconi,G., Orsenigo,F., Corada,M., Spagnuolo,R., Betson,M., Braga,V., and Dejana,E. (2002). VE-cadherin regulates endothelial actin activating Rac and increasing membrane association of Tiam. *Mol. Biol. Cell* 13, 1175-1189.
11. Xiao,K., Garner,J., Buckley,K.M., Vincent,P.A., Chiasson,C.M., Dejana,E., Faundez,V., and Kowalczyk,A.P. (2005). p120-Catenin Regulates Clathrin-dependent Endocytosis of VE-Cadherin. *Mol. Biol. Cell.*
12. Iyer,S., Ferreri,D.M., DeCocco,N.C., Minnear,F.L., and Vincent,P.A. (2004). VE-cadherin-p120 interaction is required for maintenance of endothelial barrier function. *Am. J. Physiol Lung Cell Mol. Physiol* 286, L1143-L1153.
13. Braga,V.M., Del,M.A., Machesky,L., and Dejana,E. (1999). Regulation of cadherin function by Rho and Rac: modulation by junction maturation and cellular context. *Mol. Biol. Cell* 10, 9-22.
14. Caveda,L., Martin-Padura,I., Navarro,P., Breviario,F., Corada,M., Gulino,D., Lampugnani,M.G., and Dejana,E. (1996). Inhibition of cultured cell growth by vascular endothelial cadherin (cadherin-5/VE-cadherin). *J. Clin. Invest* 98, 886-893.
15. Martin,T.A., Mansel,R., and Jiang,W.G. (2001). Hepatocyte growth factor modulates vascular endothelial-cadherin expression in human endothelial cells. *Clin. Cancer Res.* 7, 734-737.
16. Martin-Padura,I., De,C.C., Uccini,S., Pillozzi,E., Natali,P.G., Nicotra,M.R., Ughi,F., Azzolini,C., Dejana,E., and Ruco,L. (1995). Expression of VE (vascular endothelial)-cadherin and other endothelial-specific markers in haemangiomas. *J. Pathol.* 175, 51-57.
17. Hendrix,M.J., Seftor,E.A., Meltzer,P.S., Gardner,L.M., Hess,A.R., Kirschmann,D.A., Schatteman,G.C., and Seftor,R.E. (2001). Expression and functional significance of VE-cadherin in aggressive human melanoma cells: role in vasculogenic mimicry. *Proc. Natl. Acad. Sci. U. S. A* 98, 8018-8023.
18. Parker,B.S. et al. (2004). Alterations in vascular gene expression in invasive breast carcinoma. *Cancer Res.* 64, 7857-7866.
19. Shih,S.C., Robinson,G.S., Perruzzi,C.A., Calvo,A., Desai,K., Green,J.E., Ali,I.U., Smith,L.E., and Senger,D.R. (2002). Molecular profiling of angiogenesis markers. *Am. J. Pathol.* 161, 35-41.
20. Weis,S., Cui,J., Barnes,L., and Cheresh,D. (2004). Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. *J. Cell Biol.* 167, 223-229.
21. Weis,S., Cui,J., Barnes,L., and Cheresh,D. (2004). Endothelial barrier disruption by VEGF-mediated Src activity potentiates tumor cell extravasation and metastasis. *J. Cell Biol.* 167, 223-229.
22. Voura,E.B., Sandig,M., and Siu,C.H. (1998). Cell-cell interactions during transendothelial migration of tumor cells. *Microsc. Res. Tech.* 43, 265-275.
23. Liao,F. et al. (2000). Monoclonal antibody to vascular endothelial-cadherin is a potent inhibitor of angiogenesis, tumor growth, and metastasis. *Cancer Res.* 60, 6805-6810.
24. Liao,F., Doody,J.F., Overholser,J., Finnerty,B., Bassi,R., Wu,Y., Dejana,E., Kussie,P., Bohlen,P., and Hicklin,D.J. (2002). Selective targeting of angiogenic tumor vasculature by vascular endothelial-cadherin antibody inhibits tumor growth without affecting vascular permeability. *Cancer Res.* 62, 2567-2575.
25. Corada,M., Zanetta,L., Orsenigo,F., Breviario,F., Lampugnani,M.G., Bernasconi,S., Liao,F., Hicklin,D.J., Bohlen,P., and Dejana,E. (2002). A monoclonal antibody to vascular endothelial-cadherin inhibits tumor angiogenesis without side effects on endothelial permeability. *Blood* 100, 905-911.
26. Pierce,M., Wang,C., Stump,M., and Kamb,A. (2003). Overexpression of the beta-catenin binding domain of cadherin selectively kills colorectal cancer cells. *Int. J. Cancer* 107, 229-237.
27. Lim,M.J., Chiang,E.T., Hechtman,H.B., and Shepro,D. (2001). Inflammation-induced subcellular redistribution of VE-cadherin, actin, and gamma-catenin in cultured human lung microvessel endothelial cells. *Microvasc. Res.* 62, 366-382.
28. Groten,T., Kreienberg,R., Fialka,I., Huber,L., and Wedlich,D. (2000). Altered subcellular distribution of cadherin-5 in endothelial cells caused by the serum of pre-eclamptic patients. *Mol. Hum. Reprod.* 6, 1027-1032.
29. Zhang et al., 1999. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomolecular Scr.* 4:67-73

Vala Sciences [www.valasciences.com](http://www.valasciences.com) is available for contract services for analyzing lipid droplets and lipid droplet-associated proteins in human adipocytes.

For further information on products and ordering information please call or email Vala Sciences Inc, (858) 461-6862, or 888-742-8252, [info@valasciences.com](mailto:info@valasciences.com)