Infectious Angiogenesis: *Bartonella bacilliformis* Infection Results in Endothelial Production of Angiopoietin-2 and Epidermal Production of Vascular Endothelial Growth Factor

Francesca Cerimele,* Lawrence F. Brown,[†]
Francisco Bravo,[‡] Garret M. Ihler,[§]
Philomene Kouadio,[§] and Jack L. Arbiser*

From the Emory Skin Disease Research Core Center,* Department of Dermatology, Emory University School of Medicine, Boston, Massachusetts; the Department of Pathology,[†] Beth Israel Deaconess Hospital, Boston, Massachusetts; the Department of Medical Biochemistry,[§] College of Medicine, Texas A&M University System Health Science Center, College Station, Texas; and the Department of Pathology,[‡] Universidad Nacional Cayetano Heredia, Lima, Peru

Pathological angiogenesis, the development of a microvasculature by neoplastic processes, is a critical component of the development of tumors. The role of oncogenes in the induction of angiogenesis has been extensively studied in benign and malignant tumors. However, the role of infection in inducing angiogenesis is not well understood. Verruga peruana is a clinical syndrome caused by the bacterium *Bartonella bacilliformis*, and is characterized by the development of hemangioma-like lesions, in which bacteria colonize endothelial cells. To gain insight into how this bacteria induces angiogenesis *in vivo*, we performed *in situ* hybridization of clinical specimens of verruga peruana for the angiogenesis factors vascular endothelial growth factor (VEGF), its receptors VEGFR1 and VEGFR2, and angiopoietin-2. High-level expression of angiopoietin-2 and VEGF receptors was observed in the endothelium of verruga peruana. Surprisingly, the major source of VEGF production in verruga peruana is the overlying epidermis. Infection of cultured endothelium with *B. bacilliformis* also resulted in induction of angiopoietin-2 *in vitro*. These findings imply a collaboration between infected endothelium and overlying epidermis to induce angiogenesis. (Am J Pathol 2003, 163:1321–1327)

Angiogenesis, the development of microvessels, is an indispensable component of normal physiological processes, including pregnancy, menstruation, organ formation, and wound repair.^{1,2} These mechanisms have been co-opted by benign and malignant processes, as well as

inflammatory processes. Oncogenes have been shown to induce the expression of angiogenic factors including vascular endothelial growth factor (VEGF).^{3,4} Infections can also induce angiogenesis, and both viral and bacterial infections have been known to result in increased angiogenesis *in vivo*. The viral infections most commonly associated with increased angiogenesis *in vivo* include papovaviruses (SV40, human papillomavirus, polyoma), and herpesvirus (Epstein-Barr virus, human herpesvirus 8).^{5–9} These viral infections result in neoplasia and increased angiogenesis in part through viral-specific oncogenes, including SV40 large T antigen, polyoma middle T antigen, papillomavirus E6 and E7 genes, latent membrane protein 1 (LMP-1), and HHV8/KSHV-specific G proteins. The mechanisms through which bacterial infections cause angiogenesis is not well understood.¹⁰

Verruga peruana are lesions associated with human infection with *B. bacilliformis*, an arthropod transmitted disease endemic to the highlands of Peru.¹¹ Infection with *B. bacilliformis* results in Oroya fever, a severe immunosuppressive infection characterized by high fever and anemia, which has a high mortality rate if untreated.¹² This disorder is also known as Carrion's disease, after a Peruvian medical student, Daniel Carrion, who infected himself with verruga peruana tissue to prove the etiology of the disease.¹³ The convalescent state is associated with the development of hemangioma-like lesions which have been termed verruga peruana. These lesions ultrastructurally demonstrate endothelial colonization with *Bartonella* organisms.¹⁴ The mechanisms of induction of hemangioma-like lesions by *Bartonella* infection are not well understood, but has been postulated to be due to elaboration of a proangiogenic factor from infected endothelium. We demonstrate that infection of endothelium by *Bartonella* results in induction of angiopoietin-2 *in vitro* and *in vivo*. We found evidence of active angiogenesis in these lesions with high level expression of VEGFR1 and

Supported by the American Skin Association and NIAMS grant R03AR44947 (to J. L. A.), Emory Skin Disease Research Core Center grants P30 AR 42687, KO8 AR02030, and RO1 AR 47901 (to J. L. A.).

Accepted for publication June 18, 2003.

Address reprint requests to Jack L. Arbiser, Department of Dermatology, Emory University School of Medicine, WMB 5309, Atlanta, GA 30322. E-mail: jarbise@emory.edu.

Figure 1. Infection of HUVEC with *B. bacilliformis* results in induction of angiopoietin-2 mRNA. The **top bands** represent angiopoietin-2 mRNA, while the **bottom band** represents β -actin mRNA used as a loading control. I, mRNA from infected cells, NI, mRNA from non-infected control cells.

VEGFR2, which is observed in proliferative but not quiescent endothelium. However, the major source of the angiogenic factor VEGF is the suprabasal epidermis. These findings imply a collaboration between infected endothelial cells and overlying epidermis.

Materials and Methods

Infection of Human Umbilical Vein Endothelium Cell (HUVEC) with *B. bacilliformis*

HUVECs were obtained from Clonetics (San Diego, CA) and were routinely cultured in the manufacturer's own medium (EGM2) supplemented with 2% fetal bovine serum. For experimental purposes, HUVECs (passage 2 to 4) were trypsinized and seeded on 1 mg/ml gelatin, pre-coated with tissue culture flask in M199 medium (Gibco) containing 20% fetal calf serum (FCS), heparin and bovine hypothalamus extract¹⁵ the day before infection. Cultures were maintained in a humidified atmosphere 5% CO₂ and at 37°C. For infection purposes 2-day-old culture of green fluorescence protein *B. bacilliformis* were harvested, washed three times with PBS and resuspended in plain M199. The cells (about 80% confluent) were washed three times in plain M199. The cells were incubated with approximately 100 bacteria per cell in M199 medium supplemented with 10% FCS for 26 hours.¹⁶

RNA Isolation and RT-PCR

Total RNA from *Bartonella*-infected cells and non-infected cell were isolated using the TRIzol Reagent Kit (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total RNA were dissolved in RNase-free water. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using the Access RT-PCR System (Promega, Madison, WI). For angiopoietin-2 we used primers previously described with the following conditions: 1 minute at 95°C, 1 minute at 64°C, 45 seconds at 72°C for 35 cycles).^{17,18} For β -actin the following primers were used: β -actin-F, 5'-AAGATGACCCAGATCATGTTT-GAGAC-3' and β -actin-R, 5'-CTGCTTGCTGATCCACATCTGCTGG-3' (1 minute at 95°C, 1 minute at 70°C, 45 seconds at 72°C for 35 cycles). RT-PCR was performed

using the Access RT-PCR System (Promega). For angiopoietin-1 the following primers were used: hAng1F, 5'-AGTCCAGAAAAACAGTGGGAG-3' and hAng1R, 5'-AG-CAGCTGTATCTCAAGTCG-3' (1 minute at 95°C, 1 minute at 59°C, 45 seconds at 72°C for 35 cycles). For VEGF receptor 1 (VEGFR1) the following primers were used: VEGFR1F, 5'-GATGTTGAGGAAGAGGAGGATT-3' and VEGFR1R, 5'-AAGCTAGTTTCTGGGGGTATA-3' (1 minute at 95°C, 1 minute at 63°C, 45 seconds at 72°C for 35 cycles). For VEGF receptor 2 (VEGFR2) the following primers were used VEGFR2F, 5'-GATGTGGTTCT-GAGTCCGTCT-3' and VEGFR2R, 5'-CATGGCTCTGCT-TCTCCTTTG-3' (1 minute at 95°C, 1 minute at 62°C, 45 seconds at 72°C for 35 cycles).

In Situ Hybridization (ISH)

ISH was performed on 4-mm-thick sections of formalin-fixed, paraffin-embedded tissue, from four representative specimens of verruga peruana. Details of ISH have been reported previously.^{19,20} Briefly, slides were passaged through xylene and graded alcohols; 0.2 mol/L HCl, Tris/EDTA with 3 μ g/ml proteinase K/0.2% glycine/4% para-formaldehyde in phosphate-buffered saline (pH 7.4), 0.1 mol/L triethanolamine containing 1/200 (v/v) acetic anhydride, and 2X SSC. Slides were hybridized overnight at 50°C with ³⁵S-labeled riboprobes in the following mixture: 0.3 mol/L NaCl, 0.01 mol/L Tris (pH 7.6), 5 mmol/L EDTA 0.02% w/v Ficoll, 0.02% w/v polyvinylpyrrolidone, 0.02% w/v bovine serum albumin fraction V, 50% formamide, 10% dextran sulfate, 0.1 mg/ml yeast tRNA, 0.01 mol/L dithiothreitol (DTT). Post-hybridization washes included 2X SSC, 50% formamide, 10 mmol/L DTT at 50°C; 4X SSC, 10 mmol/L Tris, 1 mmol/L EDTA with 20 μ g/ml ribonuclease at 37°C; 2X SSC, 50% formamide, 10 mmol/L EDTA at 65°C; and 2X SSC. Slides were dehydrated through graded alcohols containing 0.3 mol/L ammonium acetate, dried, coated with Kodak NTB 2 emulsion and stored in the dark at 4°C for 2 weeks. The emulsion was developed with Kodak D19 developer and the slides were counterstained with hematoxylin. Antisense single-stranded ³⁵S-labeled human VPF/VEGF RNA probe and its sense control have been described previously.¹⁹ The antisense probe hybridizes specifically with a region of VPF/VEGF mRNA common to all known VPF/VEGF splice variants. ³⁵S- labeled single stranded antisense and sense RNA probes for mouse VPF/VEGF mRNA and the mouse VPF/VEGF receptors VEGFR-1 and VEGFR-2 mRNAs were described previously.¹⁹

Immunohistochemistry

Unstained sections of formalin-fixed, paraffin-embedded tissue were immunostained with polyclonal antibodies against tie-2 (clone sc-324, 1/40; Santa Cruz Biotechnologies, Santa Cruz, CA) using an avidin-biotin-complex technique with the pressure-cooker heat-induced antigen retrieval and a DAKO Autostainer (DAKO, Carpinteria, CA). Negative controls had primary antibody substituted with buffer.

Figure 2. Human verruga peruana lesions express angiopoietin-1 and -2 *in vivo*. **A:** A typical hematoxylin and eosin lesion of verruga peruana. **B:** Strong expression of angiopoietin-2 in cells surrounding lumens. **C** and **D:** Expression of tie-1 and tie-2, respectively.

Results

Bartonella infection of endothelium has been shown to result in induction of endothelial proliferation through stimulation of mediators which have not been fully characterized. Given that VEGF production by endothelium appears to be produced primarily in malignant endothelial tumors (angiosarcomas) rather than benign tumors

(hemangiomas),^{19,21} and that verruga peruana lesions are biologically benign, we felt it was unlikely that VEGF was the sole mediator of *Bartonella*-induced angiogenesis. Infection of endothelial cells with *B. bacilliformis* resulted in strong induction of angiopoietin-2 mRNA (Figure 1). Induction of other angiogenic molecules, such as VEGFR1, VEGFR2, and angiopoietin-1 were not observed (data not shown). To determine whether angiopoietin-2 is

Figure 3. Human verruga peruana lesions express tie-2 protein *in vivo*. **A:** Intense endothelial-specific expression of tie-2 in a representative verruga peruana. **B:** Negative control performed in the absence of primary antibody (magnification, $\times 40$).

expressed in authentic human verruga peruana lesions, these lesions were subjected to *in situ* hybridization for angiopoietin-2, VEGF, VEGFR1, and VEGFR2.

Prominent expression of angiopoietin-2, tie-1, and tie-2 was observed in endothelial cells of verruga peruana by *in situ* hybridization (Figure 2). In addition, prominent endothelial expression of tie-2, the receptor for angiopoietin-2, was noted by immunohistochemistry on representative sections (Figure 3). Histological analysis of four representative verruga peruana lesions revealed epidermal hyperplasia and inflammation (Figure 4, A and B). *In situ* hybridization revealed prominent expression of VEGF in the suprabasal keratinocyte layer of verruga lesions but not normal skin (Figure 4, A and B). Lesions were then examined for expression of VEGFR1 and VEGFR2, markers of angiogenic endothelium. High level expression of both VEGFR1 (Figure 4C) and VEGFR2 (Figure 4D) were observed in endothelial cells, confirming the presence of angiogenically active endothelium.

Discussion

Angiogenesis is a requirement for the maintenance of benign and malignant neoplasms, but its role in infectious disease is less well studied. While acute infection is often

associated with tissue death, chronic infection is often associated with pathological tissue overgrowth and/or infiltration of host cells. Examples of this include endothelial proliferation in verruga peruana, bacillary angiomatosis, and Kaposi's sarcoma, granuloma formation in leishmaniasis and mycobacterial disease, lymphoid proliferation in Epstein-Barr virus infection, and keratinocyte hyperplasia in human papillomavirus infection.²²

Oncogenic viruses most often encode viral-specific oncogenes which stimulate tumorigenesis and angiogenesis. In neoplasms induced by these agents, the primary source of angiogenic factors are the tumor cells themselves.^{9,23} However, in bacterial-induced angiogenesis, the source and identity of angiogenic factors are unknown. In the case of bacterial infections of endothelium, the causative organisms are often difficult to culture,²⁴ and animal models do not exist that recapitulate the pathology seen in human disease. Because of this, it is imperative to study human tissue obtained from authentic human lesions.

Prior studies of human vascular lesions have demonstrated signal transduction aberrations in benign and malignant endothelium.^{3,25,26} Increased endothelial expression of angiopoietin-2 has been observed in hemangiomas of childhood, but the role of VEGF in hemangio-

Figure 4. Expression of VEGF and VEGF receptors in verruca perwana lesions. **A** and **B**: Photomicrographs of hyperplastic epidermis overlying verruca perwana (**A**) and normal skin (**B**), showing strong expression of VEGF by suprabasal epidermal keratinocytes in verruca perwana, but low expression in normal skin (magnification, $\times 200$). **C** and **D**: Expression of VEGFR1 and VEGFR2 in verruca perwana. Photomicrographs of a nodular focus of proliferating endothelial cells and surrounding inflammatory cells showing strong expression of VEGFR1 (**C**) and VEGFR2 (**D**) by endothelial cells.

mas is controversial, with some earlier studies detecting VEGF protein expression in hemangiomas, but later studies have failed to demonstrate endothelial expression of VEGF.^{27,28} Overexpression of VEGF in endothelial cells results in malignant transformation into angiosarcoma.²¹ The prior studies suggest that endothelial expression of VEGF is a sign of malignancy.²⁹

Prior studies have implicated that *Bartonella*-infected endothelium elaborate factors which stimulate angiogenesis.³⁰ The identity of this factor(s) have not been previously elucidated. The finding of angiopoietin-2 in hemangiomas of childhood,²⁸ along with the data showing that VEGF expression in endothelium is associated with malignancy prompted us to study whether *Bartonella* in-

duces angiopoietin-2. We found that *Bartonella* infection results in induction of angiopoietin-2 *in vitro* and *in vivo*, and human verruga peruana lesions express angiopoietin-2.

The role of angiopoietin-2 in angiogenesis has not been totally elucidated. Initially angiopoietin-2 was found to have antagonistic behavior to angiopoietin-1 and bind the same receptor, tie-2.³¹ These findings led investigators to believe that angiopoietin-2 may be an inhibitor of angiogenesis. However, later findings have demonstrated that angiopoietin-2 may stimulate tumor growth, and tumor cells overexpressing angiopoietin-2 have demonstrated highly malignant behavior and leaky vessels.^{32,33} Furthermore, hemangiomas of childhood have been demonstrated to express high levels of angiopoietin-2.²⁸

Recently, *Bartonella* infection of endothelial cells *in vitro* has shown to lead to decreased apoptosis, which may account for the angiogenic phenotype.³⁴ In addition, *Bartonella* infection has been shown to cause activation of the rac GTPase, and rac GTPase activation has been shown to stimulate angiogenesis and prevent apoptosis.^{16,35,36} Consistent with these findings, angiopoietin-2 activation of tie receptor signaling has been shown to result in activation of phosphoinositol-3 kinase/akt signaling, which are associated with both protection against apoptosis and stimulation of endothelial proliferation.^{37,38}

Interestingly, we found that overlying epidermis is the primary source of VEGF in our lesions, and may serve as a trophic factor to maintain hemangioma-like lesions induced by *Bartonella* infection. Epidermal production of VEGF appears to be a common response to inflammatory stimuli in the skin, and may be due to elaboration of cytokines by the inflammatory process³⁹ or through disruption of the epidermis by the verruga peruana lesions. Barrier disruption of the epidermis leads to induction of VEGF (Cerimele and Arbiser, unpublished data). We have observed that verruga peruana specimens express high levels of active mitogen-activated protein kinase (P-MAPK), as do hemangiomas of childhood.⁴⁰ In contrast, malignant endothelial lesions (angiosarcoma), express low levels of activated MAP kinase, reflecting a decreased requirement for MAP kinase in tumorigenesis.^{40,41} Verruga peruana are highly angiogenic lesions, but are histologically benign. This is a fundamentally different pattern of angiogenesis compared with either virally-induced tumors or angiosarcomas (malignant endothelial tumors) in which the tumor cells themselves are the source of angiogenic factors.^{19,21,42} Our finds point to a novel form of angiogenesis in benign lesions, in which proliferation of endothelium and/or decreased apoptosis may be due to autocrine loops of angiopoietin-2 and tie-2, with paracrine contributions of VEGF produced outside the infected endothelium. The paracrine expression of VEGF may help maintain the lesions.

Bartonella infections are unique among bacteria in induction of benign hemangioma-like lesions. Currently, verruga peruana is an uncommon disease, but it histologically resembles the benign hemangioma of infancy, the most common neoplasm of children. This report is the first demonstration of a potential role of angiopoietin-2 in

the pathogenesis of *Bartonella* infection, and demonstrates a novel mechanism of bacterial induction of angiogenesis.

References

1. Klauber N, Rohan RM, Flynn E, D'Amato RJ: Critical components of the female reproductive pathway are suppressed by the angiogenesis inhibitor AGM-1470. *Nat Med* 1997, 3:443-446
2. Folkman J: Seminars in Medicine of the Beth Israel Hospital, Boston: clinical applications of research on angiogenesis. *N Engl J Med* 1995, 333:1757-1763
3. Arbiser JL, Moses MA, Fernandez CA, Ghiso N, Cao Y, Klauber N, Frank D, Brownlee M, Flynn E, Parangi S, Byers HR, Folkman J: Oncogenic H-ras stimulates tumor angiogenesis by two distinct pathways. *Proc Natl Acad Sci USA* 1997, 94:861-866
4. Rak J, Mitsuhashi Y, Sheehan C, Tamir A, Viloria-Petit A, Filmus J, Mansour SJ, Ahn NG, Kerbel RS: Oncogenes and tumor angiogenesis: differential modes of vascular endothelial growth factor up-regulation in ras-transformed epithelial cells and fibroblasts. *Cancer Res* 2000, 60:490-498
5. Carbone M, Pass HI, Rizzo P, Marinetti M, Di Muzio M, Mew DJ, Levine AS, Procopio A: Simian virus 40-like DNA sequences in human pleural mesothelioma. *Oncogene* 1994, 9:1781-1790
6. Flore O, Rafii S, Ely S, O'Leary JJ, Hyjek EM, Cesarman E: Transformation of primary human endothelial cells by Kaposi's sarcoma-associated herpesvirus. *Nature* 1998, 394:588-592
7. Majewski S, Szmurlo A, Marczak M, Jablonska S, Bollag W: Synergistic effect of retinoids and interferon- α on tumor-induced angiogenesis: anti-angiogenic effect on HPV-harboring tumor-cell lines. *Int J Cancer* 1994, 57:81-85
8. Arbeit JM, Olson DC, Hanahan D: Up-regulation of fibroblast growth factors and their receptors during multi-stage epidermal carcinogenesis in K14-HPV16 transgenic mice. *Oncogene* 1996, 13:1847-1857
9. Lopez-Ocejo O, Viloria-Petit A, Bequet-Romero M, Mukhopadhyay D, Rak J, Kerbel RS: Oncogenes and tumor angiogenesis: the HPV-16 E6 oncoprotein activates the vascular endothelial growth factor (VEGF) gene promoter in a p53 independent manner. *Oncogene* 2000, 19:4611-4620
10. Arbiser JL: Angiogenesis and the skin: a primer. *J Am Acad Dermatol* 1996, 34:486-497
11. Spach DH, Koehler JE: Bartonella-associated infections. *Infect Dis Clin North Am* 1998, 12:137-155
12. Caceres-Rios H, Rodriguez-Tafur J, Bravo-Puccio F, Maguina-Vargas C, Diaz CS, Ramos DC, Patarca R: Verruga peruana: an infectious endemic angiomatosis. *Crit Rev Oncog* 1995, 6:47-56
13. Sutton J: Daniel Carrion and Oroya fever. *Med J Aust* 1971, 2:589-590
14. Verma A, Davis GE, Ihler GM: Formation of stress fibres in human endothelial cells infected with *Bartonella bacilliformis* is associated with altered morphology, impaired migration and defects in cell morphogenesis. *Cell Microbiol* 2001, 3:169-180
15. Friesel RE, Maciag T: Molecular mechanisms of angiogenesis: fibroblast growth factor signal transduction. *EMBO J* 1995, 9:919-925
16. Verma A, Davis GE, Ihler GM: Infection of human endothelial cells with *Bartonella bacilliformis* is dependent on Rho and results in activation of Rho. *Infect Immun* 2000, 68:5960-5969
17. Yu Q, Stamenkovic I: Angiopoietin-2 is implicated in the regulation of tumor angiogenesis. *Am J Pathol* 2001, 158:563-570
18. Pola R, Ling LE, Silver M, Corbly MJ, Kearney M, Blake PR, Shapiro R, Taylor FR, Baker DP, Asahara T, Isner JM: The morphogen Sonic hedgehog is an indirect angiogenic agent upregulating two families of angiogenic growth factors. *Nat Med* 2001, 7:706-711
19. McLaughlin ER, Brown LF, Weiss SW, Mulliken JB, Perez-Atayde A, Arbiser JL: VEGF and its receptors are expressed in a pediatric angiosarcoma in a patient with Aicardi's syndrome. *J Invest Dermatol* 2000, 114:1209-1210
20. Claffey KP, Brown LF, del Aguila LF, Tognazzi K, Yeo KT, Manseau EJ, Dvorak HF: Expression of vascular permeability factor/vascular endothelial growth factor by melanoma cells increases tumor growth, angiogenesis, and experimental metastasis. *Cancer Res* 1996, 56:172-181

21. Arbiser JL, Larsson H, Claesson-Welsh L, Bai X, LaMontagne K, Weiss SW, Soker S, Flynn E, Brown LF: Overexpression of VEGF 121 in immortalized endothelial cells causes conversion to slowly growing angiosarcoma and high-level expression of the VEGF receptors VEGFR-1 and VEGFR-2 in vivo. *Am J Pathol* 2000, 156:1469–1476
22. Koehler JE, Quinn FD, Berger TG, LeBoit PE, Tapper JW: Isolation of *Rochalimaea* species from cutaneous and osseous lesions of bacillary angiomatosis. *N Engl J Med* 1992, 327:1625–1631
23. Weninger W, Uthman A, Pammer J, Pichler A, Ballaun C, Lang IM, Plettenberg A, Bankl HC, Sturzl M, Tschachler E: Vascular endothelial growth factor production in normal epidermis and in benign and malignant epithelial skin tumors. *Lab Invest* 1996, 75:647–657
24. Garcia FU, Wojta J, Broadley KN, Davidson JM, Hoover RL: *Bartonella bacilliformis* stimulates endothelial cells in vitro and is angiogenic in vivo. *Am J Pathol* 1990, 136:1125–1135
25. Bielenberg DR, Bucana CD, Sanchez R, Mulliken JB, Folkman J, Fidler IJ: Progressive growth of infantile cutaneous hemangiomas is directly correlated with hyperplasia and angiogenesis of adjacent epidermis and inversely correlated with expression of the endogenous angiogenesis inhibitor, IFN- β . *Int J Oncol* 1999, 14:401–408
26. North PE, Waner M, Mizeracki A, Mihm MC Jr: GLUT1: a newly discovered immunohistochemical marker for juvenile hemangiomas. *Hum Pathol* 2000, 31:11–22
27. Takahashi K, Mulliken JB, Kozakewich HP, Rogers RA, Folkman J, Ezekowitz RA: Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 1994, 93:2357–2364
28. Boye E, Yu Y, Paranya G, Mulliken JB, Olsen BR, Bischoff J: Clonality and altered behavior of endothelial cells from hemangiomas. *J Clin Invest* 2001, 107:745–752
29. Brown LF, Dezube BJ, Tognazzi K, Dvorak HF, Yancopoulos GD: Expression of Tie1, Tie2, and angiopoietins 1, 2, and 4 in Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol* 2000, 156:2179–2183
30. Garcia FU, Wojta J, Hoover RL: Interactions between live *Bartonella bacilliformis* and endothelial cells. *J Infect Dis* 1992, 165:1138–1141
31. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, Compton D, McClain J, Aldrich TH, Papadopoulos N, Daly TJ, Davis S, Sato TN, Yancopoulos GD: Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. *Science* 1997, 277:55–60
32. Tanaka S, Mori M, Sakamoto Y, Makuuchi M, Sugimachi K, Wands JR: Biologic significance of angiopoietin-2 expression in human hepatocellular carcinoma. *J Clin Invest* 1999, 103:341–345
33. Zagzag D, Hooper A, Friedlander DR, Chan W, Holash J, Wiegand SJ, Yancopoulos GD, Grumet M: In situ expression of angiopoietins in astrocytomas identifies angiopoietin-2 as an early marker of tumor angiogenesis. *Exp Neurol* 1999, 159:391–400
34. Kirby JE, Nekorchuk DM: *Bartonella*-associated endothelial proliferation depends on inhibition of apoptosis. *Proc Natl Acad Sci USA* 2002, 99:4656–4661
35. Eriksson A, Cao R, Roy J, Tritsarlis K, Wahlestedt C, Dissing S, Thyberg J, Cao Y: Small GTP-binding protein Rac is an essential mediator of vascular endothelial growth factor-induced endothelial fenestrations and vascular permeability. *Circulation* 2003, 107:1532–1538
36. Jiang K, Zhong B, Ritchey C, Gilvary DL, Hong-Geller E, Wei S, Djeu JY: Regulation of Akt-dependent cell survival by Syk and Rac. *Blood* 2003, 101:236–244
37. Kim I, Kim JH, Moon SO, Kwak HJ, Kim NG, Koh GY: Angiopoietin-2 at high concentration can enhance endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. *Oncogene* 2000, 19:4549–4552
38. Zagzag D, Amirnovin R, Greco MA, Yee H, Holash J, Wiegand SJ, Zabski S, Yancopoulos GD, Grumet M: Vascular apoptosis and involution in gliomas precede neovascularization: a novel concept for glioma growth and angiogenesis. *Lab Invest* 2000, 80:837–849
39. Schon MP, Detmar M, Parker CM: Murine psoriasis-like disorder induced by naive CD4+ T cells. *Nat Med* 1997, 3:183–188
40. Arbiser JL, Weiss SW, Arbiser ZK, Bravo F, Govindajaran B, Caceres-Rios H, Cotsonis G, Recavarren S, Swerlick RA, Cohen C: Differential expression of active mitogen-activated protein kinase in cutaneous endothelial neoplasms: implications for biologic behavior and response to therapy. *J Am Acad Dermatol* 2001, 44:1–5
41. LaMontagne Jr KR Jr., Moses MA, Wiederschain D, Mahajan S, Holden J, Ghazizadeh H, Frank DA, Arbiser JL: Inhibition of MAP kinase causes morphological reversion and dissociation between soft agar growth and in vivo tumorigenesis in angiosarcoma cells. *Am J Pathol* 2000, 157:1937–1945
42. Brown LF, Tognazzi K, Dvorak HF, Harrist TJ: Strong expression of kinase insert domain-containing receptor, a vascular permeability factor/vascular endothelial growth factor receptor in AIDS-associated Kaposi's sarcoma and cutaneous angiosarcoma. *Am J Pathol* 1996, 148:1065–1074