Big Endothelin-1 and Interleukin-6 Modulation in Human Microvascular Endothelial Cells After Human Herpesvirus 8 Infection

LIVIANNA SPECIALE,* RENATO BIFFI,* ROBERTA MANCUSO,* ELISA BORGHI,* ROMINA MAZZIOTTI,* AND PASQUALE FERRANTE*,†,[1]

Laboratory of Molecular Medicine and Biotechnology, Don C. Gnocchi Foundation, IRCCS, Via Capecelatro 66, 20148 Milan, Italy, and †Chair of Virology Department of Biomedical Science and Technology, University of Milan, Italy

Endothelin (ET)-1 is an angiogenic factor that, among others, is secreted by endothelial cells during development of several neoplasias. In particular, Kaposi sarcoma (KS) skin lesions show overexpression of the ET-1 system. Spindle cells, which characterize tumor lesions, are of endothelial origin and during disease are infected by human herpesvirus 8 (HHV-8). The majority of these cells are latently infected, suggesting that latent genes are sufficient for maintenance of viral infection and development of KS. The establishment of a reliable infection system is required to better understand the role of viral and cellular angiogenic factors involved in KS progression. For this purpose, we used human microvascular endothelial cells (HMEC-1) to establish an ET-1–producing model of infection with HHV-8. Viral particles purified from BCBL-1 cells were used to infect HMEC-1 monolayer, and infection was assessed by polymerase chain reaction, reverse transcription polymerase chain reaction, and confocal microscopy. Mitochondrial activity and cell viability, measured at 24, 48, and 72 hours after infection by 3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, was reduced in HHV-8–infected cells compared with control. In contrast, 1 week after infection, HHV-8–positive cells showed higher mitochondrial functionality. Endothelin production was measured in culture media collected at 24, 48, and 72 hours after infection. The levels of endothelin precursor big endothelin-1 was increased 3 days after infection, although big ET-1 and ET-1 production did not differ significantly between infected and uninfected cells. These results indicate this model as a useful tool to further characterize the effects of HHV-8 in the early and late phases of infection, and to determine its ability to interfere with the endothelin system. Exp Biol Med 231:1171–1175, 2006

Key words: big ET-1; ET-1; IL-6; Kaposi sarcoma; HHV-8

Introduction

Endothelin (ET)-1 is a vascular peptide with angiogenic functions produced by endothelial cells (1). Aberrant ET-1–induced cell proliferation or survival of tumor cells are implicated in the pathogenesis of many malignancies, including Kaposi sarcoma (KS) (2–5). Infection with human herpesvirus 8 (HHV-8) is closely associated with the development of KS (6), a multifocal vascular neoplasm involving the skin, visceral organs, and lymph nodes, and has also been identified in primary effusion lymphoma (7) and multicentric Castleman disease (8). KS skin lesions, at different stages of development, show overexpression of the ET-1 system, including receptors and peptides (9, 10), and are characterized by the presence of spindle cells, a population of endothelial origin infected with HHV-8 (11). Although lytic replication is required for virus production, the majority of spindle cells are latently infected, suggesting that latent genes are sufficient for maintenance of viral infection. In this study, we established an *in vitro* model using human microvascular endothelial cells (HMEC-1) to analyze the early phase of HHV-8 infection. Indeed, HHV-8–infected endothelial cells are detected in the initial phases of the disease before spindle cell formation, which might be a consequence of endothelial cell infection (12). Therefore, KS lesions could be the result of paracrine interactions between its cellular components: the production of inflammatory and angiogenic factors by spindle cells and the ability of inflammatory cells to produce cytokines may synergistically contribute to promote spindle

Supported by a grant from 2005 Ricerca Corrente (Italian Ministry of Health) assigned to Don Gnocchi Foundation, IRCCS.

[1] To whom correspondence should be addressed at Laboratory of Molecular Medicine and Biotechnology, Don C. Gnocchi Foundation, IRCCS, Via Capecelatro 66, 20148 Milan, Italy. E-mail: pferrante@dongnocchi.it

Received September 23, 2005.
Accepted November 17, 2005.

1535-3702/06/2316-1171$15.00

cells survival and growth. In early-stage KS, several inflammatory cells are recruited into KS lesions. These cells produce high levels of proinflammatory cytokines, including interleukin (IL)-6, tumor necrosis factor-α, IL-1α, and IL-1β (13). *In vitro* evidence indicates that IL-6 contributes to the pathogenesis of KS. In fact, IL-6 and other cytokines such as IL-8, basic fibroblastic growth factor, and vascular endothelial growth factor have a critical role for spindle cells proliferation, endothelial cell migration and for KS lesions development *in vivo* (14, 15).

Although the majority of KS endothelial and spindle cells are infected with HHV-8, the establishment of cell lines able to maintain the infection *in vitro* displayed several obstacles. Indeed, KS-derived cell lines either have limited life or lose the viral genome in a few passages (16). Therefore, the establishment of a reliable infection system is required to better understand the role of viral and cellular angiogenic factors involved in KS progression.

In particular, the aim of our study was to obtain a stably HHV-8–infected endothelial cell line and analyze, early after infection, the mitochondrial activity and the production of big ET-1, ET-1, and IL-6 by HMEC-1.

Materials and Methods

Cell Cultures. Human microvascular endothelial cell line-1 immortalized by simian virus 40 large T antigen was kindly provided by Dr. F. J. Candal (Atlanta, GA). Cells were maintained in complete medium constituted by MCDB 131 (GIBCO, Paisley, Scotland) supplemented with 10% fetal calf serum (Hyclone, Celbio, Pero, Italy), 10 ng/ml epidermal growth factor (PeproTech EC, London, UK), 1 μg/ml hydrocortisone (Sigma Italia, Milan, Italy), 2 m*M* glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 20 m*M* Hepes buffer (Euroclone, Celbio).

HHV-8–positive, Epstein Barr virus-negative BCBL-1 cell line was maintained in RPMI medium (Euroclone, Celbio) supplemented with 10% fetal bovine serum, 2 m*M* glutamine, 100 IU/ml penicillin, and 100 μg/ml streptomycin.

Virus Purification from BCBL-1 cells. Latently infected BCBL-1 cells were stimulated to enter the viral lytic cycle by addition of 20 ng/ml of 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Sigma Italia, Milan, Italy) and 200 U/ml of hIL-6 (PeproTech EC) in the culture media. After 96 hours the supernatants were separated by centrifugation, filtered through a 0.45 μm filter and ultracentrifuged at 17000 rpm (rotor JA17, Beckman Instruments, Palo Alto, CA) for 4 hours at 4°C. The pellet was finally resuspended in 2 ml of MCDB 131 complete medium and stored at −80°C.

Infectious particles were titered on HMEC-1 cells to determine the optimal amount of virus needed for infection in 80% confluent 25 cm^2 flask or 12-well plates.

Infection of HMEC-1 with HHV-8. Viral particles partially purified from BCBL-1 cells were added to 2×10^5 confluent monolayers of HMEC-1 in 12-well, flat-bottom microplates (Costar Italia, Milan, Italy). After an incubation of 2 hours at 37°C, fresh medium was added to infected cells. Uninfected HMEC-1 were used as controls. Cells were incubated for 24, 48, or 72 hours at 37°C in 5% CO_2 in presence or absence of 100 U/ml hIL-6 (PeproTech EC). For some experiments, cells were collected after 72 hours by trypsin-EDTA treatment, washed, and plated in 75 cm^2 flask and maintained for 3 days. At Day 6 after infection, the cells were trypsin-EDTA treated and plated into 12-well, flat-bottom microplates for 3 additional days (i.e., 7, 8, and 9 days from the infection). At the end of treatment, the supernatants were collected and tested for the presence of ET-1, big ET-1, or IL-6.

DNA Polymerase Chain Reaction. Infected HMEC-1 cells were tested for the presence of HHV-8 DNA by nested polymerase chain reaction (n-PCR) of the kaposin region of the viral genome using the following primers: OK12F (5′-CTTTGGGAGGGCACGCTAGCT-3′) and OK12R (5′-TCCTCACTCCAATCCCAATGC-3′) for the first step and NK12F (5′-CAACTCGTGTCCT-GAATGCT-3′) and NK12R (5′-GGTGTTTGTGGCAGTT-CATG-3′) for the second step. Both reactions were performed with 30 cycles at annealing temperature (Ta), respectively, of 62°C and 60°C, allowing the amplification of a final 148-bp fragment.

Confocal Microscopy. Cells seeded on chambers slides (Nalge Nunc International, Rochester, NY) were fixed in 4% paraformaldehyde for 15 minutes at room temperature and permeabilized with 0.1% Triton X-100. After blocking with 1% bovine serum albumin in phosphate-buffered saline for 30 minutes, latent nuclear antigen (LANA; Advanced Biotechnologies, Columbia, MD) expression was detected by confocal microscopy (TCS SP2; Leica Microsystems, Wetzlar, Germany) using 1:200 dilution of rat monoclonal antibody directed against LANA and anti-rat FITC-conjugated secondary antibody (Sigma Italia).

Reverse Transcription PCR. Total RNA was extracted from infected and uninfected HMEC-1 cells by QIAamp RNA Blood kit (Qiagen Italia, Milan, Italy). Residual DNA contamination was eliminated by digestion with DNAse I (Ambion, Austin, TX). One hundred nanograms of total RNA were used for retrotranscription reaction, followed by cDNA amplification using Superscript One-step RT-PCR kit (Invitrogen Italia, Milan, Italy) with the same primers shown previously for the kaposin region and the following primers for the amplification of ORF73 region (LANA): O73F (5′-AGGAGCAGGAGACGGTGG-3′), O73R (5′-TTCCTGTGGAGAGTCCCCA-3′), N73F (5′-CATCCAAGATACCAACAACC-3′), and N73R (5′-ATAAGTTATGGGCGACTGGT-3′). Retrotranscription reaction at 45°C for 30 mins and 55°C for 30 mins steps was followed by two steps of amplification (35 cycles each, at Ta of 56°C) to obtain a final product of 267 bp.

3,(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide (MTT) Assay and Cell Viability. Vi-

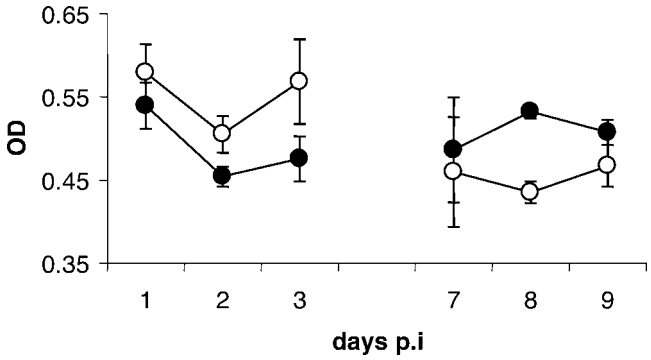

Figure 1. Mitochondrial activity by MTT assay performed on infected (●) or uninfected (○) HMEC-1 cells for 9 days. Each time point is expressed in optical density and represents the mean ± SEM of six different experiments performed in duplicate.

able cells were detected by direct counting with trypan blue exclusion. To assay mitochondrial activity, for all the experiments at the end of treatments, the supernatants were collected and adherent cells analyzed by MTT colorimetric assay of Mosmann as previously described (17). Briefly, the cells were incubated with 500 μl of complete medium containing 50 μg/ml MTT. After 2 hours, the supernatants were discarded and crystals dissolved in 500 μl of sodium dodecyl sulfate 20% in dimethyl formamide 50%. After dissolving the dark blue formazan crystals, the plates were read in a microplate reader using a test wavelength of 550 nm and a reference wavelength of 620 nm.

Big ET-1, ET-1, and IL-6 Assay. The levels of big ET-1 or ET-1 in the supernatants of both infected and uninfected endothelial cells were analyzed using the human big ET-1 or ET-1 enzyme immunometric assay kit (Assay Designs, Inc., Ann Arbor, MI), following the manufacturer's procedures. The sensitivities of the kits are 0.14 pg/ml or 0.23 pg/ml respectively. Interleukin-6 levels were assayed using the human IL-6 enzyme linked assay kit (R&D System Inc. Minneapolis, MN). The minimal detectable dose of IL-6 is less than 0.70 pg/ml.

Statistical Analysis. All the tests were performed at least four times in duplicate. The data are presented as means ± standard error mean (SEM). Student's *t* test was employed in the statistical analysis. Differences with P value < 0.05 were considered significant.

Results

HMEC-1 Infection with HHV-8. Viral particles partially purified from stimulated BCBL-1 cells were used to infect endothelial cells monolayer and viral DNA was searched in infected cells by nested PCR of the kaposin region. After DNA extraction and PCR reaction, cells treated with HHV-8 particles resulted positive (whereas uninfected cells did not) for the presence of the viral genome (data not shown).

Reverse transcriptase-PCR was performed to identify the transcriptional activity of HHV-8 by amplification of the kaposin (data not shown) and LANA regions of the viral genome (data not shown). Moreover the expression of latency associated nuclear antigen was determined by confocal microscopy using a specific monoclonal antibody (data not shown). In both cases, results were consistent in different experiments, demonstrating that cells are successfully infected after incubation with HHV-8 particles. In addition, the presence of viral DNA and the expression of viral proteins after several passages (up to 2 months after infection) demonstrate the establishment of a persistent infection in this cell line.

Evaluation of Mitochondrial Activity and Cell Proliferation. When we compared HMEC-1 infected and uninfected cells, we observed, as shown in Fig. 1, that at the first day after infection, and until Day 3 after infection, mitochondrial activity of HHV-8–positive cells was reduced. However, when the cells were cultured for more than 1 week, we observed a reduction of MTT activity in uninfected controls, whereas in HHV-8–positive cells, mitochondrial activity was stable. Indeed at Days 7, 8, and 9 after infection, mitochondrial activity was higher in infected cells when compared with control, although the difference was not statistically significant. Moreover, when cell proliferation was determined counting viable cells by trypan blue 3 weeks after infection, HHV-8–infected cells were growing faster than controls and no morphologic change was noticed (data not shown).

Big ET-1 and ET-1 Production by HMEC-1. When HMEC-1 monolayer was cultured in the presence of HHV-8, a significant increase was observed ($P < 0.05$) in big ET-1 production on Day 3 (33.5 ± 4.73 pg/ml) compared with the first day after infection (17.0 ± 3.05 pg/ml), whereas big ET-1 production was stable in uninfected cells. Moreover, we noticed higher levels of big ET-1 secreted from infected cells after 3 days from infection compared with controls, although not statistically significant (Fig. 2A). Big ET-1 production in both infected and uninfected cells did not show any significant difference when cells were cultured in presence or in absence of IL-6 (data not shown).

On the first day of culture, high levels of ET-1 were present in the supernatants of HMEC-1 cells infected (62.8 ± 8 pg/ml) or uninfected (63.9 ± 5.55 pg/ml) (Fig. 2B), both in presence and absence of IL-6 (data not shown). Nevertheless we observed a gradual decrease of ET-1 to 25.6 ± 4.62 pg/ml 72 hours p.i. in any conditions (Fig. 2B).

We are performing additional experiments to analyze the levels of ET-1 and big ET-1 released in the supernatants during the later phases of infection (more than 3 days after infection), but no definitive data are available at this time.

IL-6 Production by HMEC-1. In our experiments, we observed that the production of IL-6 by HMEC-1–infected cells was already evident at the first day after infection, showing a 6-fold increase in the levels of this cytokine in HHV-8–positive cells (57.4 ± 19.35 pg/ml) compared with uninfected cells (9.3 ± 3.4 pg/ml) (Fig. 3).

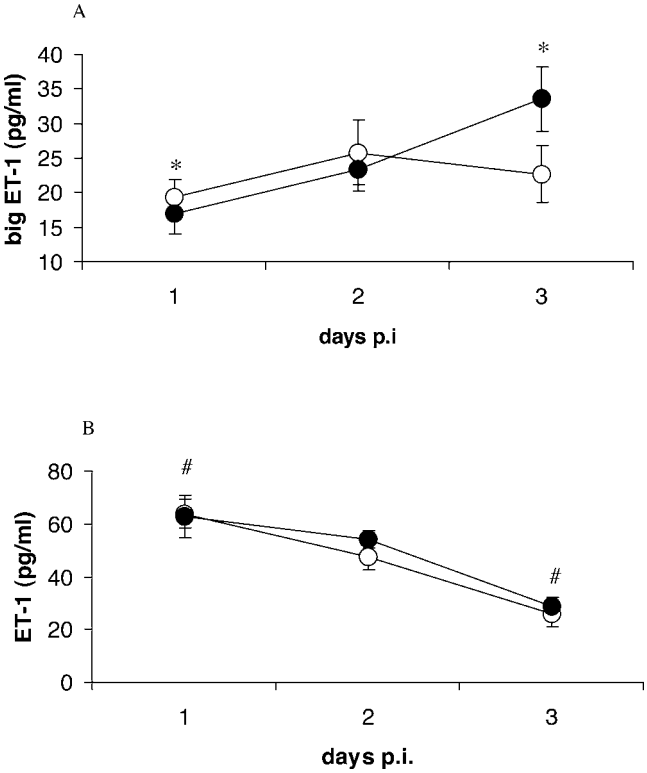

Figure 2. Big ET-1 (A) and ET-1 (B) production by HHV-8 infected (●) or uninfected (○) HMEC-1 cells. Each point is expressed in pg/ml and represents the mean ± SEM of four experiments performed in duplicate. * $P < 0.05$ between HHV-8 infected cells. (●) Day 3 versus Day 1 after infection. # $P < 0.001$ between HHV-8–infected (●) and -uninfected (○) cells Day 3 versus Day 1 after infection.

IL-6 release was stable during the course of infection until Day 3 after infection; in all time points, the differences between infected and uninfected cells were statistically significant (Fig. 3).

We performed the same set of experiments after the infection for 9 days and observed that IL-6 production in infected cells was more highly preserved than in uninfected cells (data not shown).

Discussion

In the early stages of KS, lesions appear reactive and are stimulated to grow by the action of inflammatory cytokines and growth factors. Infection with HHV-8 seems to be necessary but not sufficient to develop KS, and perturbations in the level and activity of some cytokines and vascular mediators seem to contribute to develop and amplify the lesions (18). In our results, HHV-8 negatively affected cellular functions during the first days of infection in HMEC-1 cell line, and in particular mitochondrial activity. In addition, after 1 week, HHV-8–infected cells showed stable cellular activity and proliferation, whereas in uninfected cells, mitochondrial functions were reduced. These observations seem to suggest that viral proteins might influence host cell physiology by targeting mitochondria and resulting in an increased cell proliferation (19).

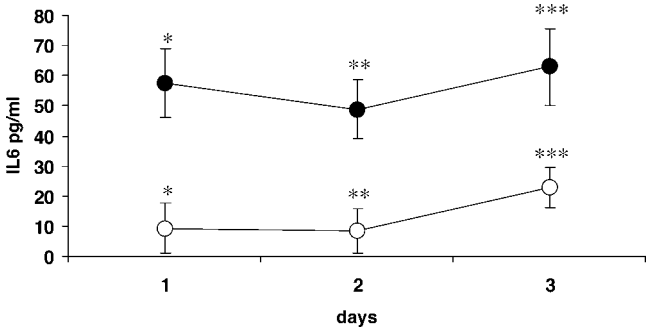

Figure 3. Kinetics of IL-6 production by HMEC-1 cells cultured in presence (●) and in absence (○) of HHV-8. Results are expressed in pg/ml and represent the mean ± SEM of three experiments performed in duplicate. * $P < 0.02$ between HHV-8–infected (●) or -uninfected (○) HMEC-1 cells. ** $P = 0.03$ between HHV-8–infected (●) or -uninfected (○) HMEC-1 cells. *** $P < 0.01$ between HHV-8–infected (●) or -uninfected (○) HMEC-1 cells.

Different angiogenic molecules are produced during the progression of KS lesions, including ET-1 that is increased in other tumors or in the initiation of pulmonary hypertension after HHV-8 infection (2, 21). ET-1 is a small peptide produced constitutively by endothelial cells through cleavage of big ET-1 by endothelin-converting enzyme (ECE) (1). ET-1 levels are overexpressed in many tumors (20) and act as an autocrine growth factor on endothelial cells (9). In our experimental model, big ET-1 production by HHV-8–infected HMEC-1 cells increased during the course of infection. On Day 3 after infection, we observed a statistically significant difference of big ET-1 release compared with the first day after infection. Surprisingly, in our experiments, the levels of ET-1 gradually decreased in both infected and uninfected cells leading to an accumulation of ET-1 precursor during the first phase of infection. Even if up to date, we do not have enough data to confirm this hypothesis; these observations could be the consequence of ECE recycle from cell surface to the cytoplasm or the effect of early viral proteins on ECE activity. To further understand the mechanism driving ECE activity and ET-1 production, additional studies aimed to look at mRNA expression will be performed.

Nevertheless this observation raises the possibility that big ET-1, together with ET-1, may contribute to lesions development in KS pathogenesis. In addition, because ET receptor antagonism is a new therapeutic approach for pulmonary hypertension, our data might contribute to better understand the role of HHV-8 infection in pulmonary hypertension progression (21).

In early KS lesions it was observed that a large number of infiltrating cells producing IL-6, IL-1, and tumor necrosis factor-α can mediate KS spindle cells growth (18, 22) or tumor cell infiltration. Moreover, previous studies *in vitro* demonstrated that viral proteins such as LANA and viral FLICE inhibitory protein (vFLIP) induce the activation of cellular IL-6 promoter (23). In addition, high levels of IL-6

(22) and specific IL-6 promoter genotypes have been indicated as risk factors for the development of KS (14).

Our data point out that IL-6 production is stimulated by HHV-8 already 24 hours after infection. Although HHV-8 expresses an IL-6 homolog (vIL-6), our detection method is specific for human IL-6 and does not recognize the viral protein. Moreover the levels of IL-6 released by infected cells are stable for at least 1 week and are significantly higher than those produced by uninfected cells. Therefore, our results indicate that IL-6 is potentially important during the early phase of HHV-8 infection in HMEC-1 cells.

Based on these findings, we could hypothesize that the early phases of KS lesions development could be related to the presence of appropriate inflammatory signals, already present in tumor environment. Because the relationship between viral components, endothelins, and cytokines is still not clear, further studies will be conducted to clarify the mechanisms of KS pathogenesis.

1. Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T. A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415, 1988.
2. Nelson J, Bagnato A, Battistini B, Nisen P. The endothelin axis: emerging role in cancer. Nat Rev Cancer 3:110–116, 2003.
3. Kopetz ES, Nelson JB, Carducci MA. Endothelin-1 as a target for therapeutic intervention in prostate cancer. Invest New Drugs 20:173–182, 2002.
4. Bagnato A, Tecce R, Moretti C, Di Castro V, Spergel D, Catt KJ. Autocrine actions of endothelin-1 as a growth factor in human ovarian carcinoma cells. Clin Cancer Res 1:1059–1066, 1995.
5. Bagnato A, Rosano L, Di Castro V, Albini A, Salani D, Varmi M, Nicotra MR, Natali PG. Endothelin receptor blockade inhibits proliferation of Kaposi's sarcoma cells. Am J Pathol 158:841–847, 2001.
6. Chang Y, Cesarman E, Pessin MS, Lee F, Culpepper J, Knowles DM, Moore PS. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. Science 266:1865–1869, 1994.
7. Cesarman E, Chang Y, Moore PS, Said JW, Knowles DM. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. N Engl J Med 332:1186–1191, 1995.
8. Soulier J, Grollet L, Oksenhendler E, Cacoub P, Cazals-Hatem D, Babinet P, d'Agay MF, Clauvel JP, Raphael M, Degos L, Sigaux F. Kaposi's sarcoma-associated herpesvirus-like DNA sequences in multicentric Castleman's disease. Blood 86:1276–1280, 1995.
9. Salani D, Taraboletti G, Rosano L, Di Castro V, Borsotti P, Giavazzi R, Bagnato A. Endothelin-1 induces an angiogenic phenotype in cultured endothelial cells and stimulates neovascularization in vivo. Am J Pathol 157:1703–1711, 2000.
10. Rosano L, Spinella F, Di Castro V, Nicotra MR, Albini A, Natali PG, Bagnato A. Endothelin receptor blockade inhibits molecular effectors of Kaposi's sarcoma cell invasion and tumor growth in vivo. Am J Pathol 163:753–762, 2003.
11. Staskus KA, Zhong W, Gebhard K, Herndier B, Wang H, Renne R, Beneke J, Pudney J, Anderson DJ, Ganem D, Haase AT. Kaposi's sarcoma-associated herpesvirus gene expression in endothelial (spindle) tumor cells. J Virol 71:715–719, 1997.
12. Moses AV, Fish KN, Ruhl R, Smith PP, Strussenberg JG, Zhu L, Chandran B, Nelson JA. Long-term infection and transformation of dermal microvascular endothelial cells by human herpesvirus 8. J Virol 73:6892–6902, 1999.
13. Brockmeyer NH, Willers CP, Anders S, Mertins L, Rockstroh JK, Sturzl M. Cytokine profile of HIV-positive Kaposi's sarcoma derived cells in vitro. Eur J Med Res 4:95–100, 1999.
14. Foster CB, Lehrnbecher T, Samuels S, Stein S, Mol F, Metcalf JA, Wyvill K, Steinberg SM, Kovacs J, Blauvelt A, Yarchoan R, Chanock SJ. An IL-6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood 96:2562–2567, 2000.
15. Masood R, Cai J, Tulpule A, Zheng T, Hamilton A, Sharma S, Espina BM, Smith DL, Gill PS. Interleukin 8 is an autocrine growth factor and a surrogate marker for Kaposi's sarcoma. Clin Cancer Res 7:2693–2702, 2001.
16. Bechtel JT, Liang Y, Hvidding J, Ganem D. Host range of Kaposi's sarcoma-associated herpesvirus in cultured cells. J Virol 77:6474–6481, 2003.
17. Mosman T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assay. J Immunol Methods 65:55–63, 1983.
18. Ensoli B, Sturzl M. Kaposi's sarcoma: a result of the interplay among inflammatory cytokines, angiogenic factors and viral agents (review). Cytokine Growth Factor Rev 9:63–83, 1998.
19. D'Agostino DM, Bernardi P, Chieco-Bianchi L, Ciminale V. Mitochondria as functional targets of proteins coded by human tumor viruses (review). Adv Cancer Res 94:87–142, 2005.
20. Bagnato A, Salani D, Di Castro V, Wu-Wong JR, Tecce R, Nicotra MR, Venuti A, Natali PG. Expression of endothelin 1 and endothelin A receptor in ovarian carcinoma: evidence for an autocrine role in tumor growth. Cancer Res 59:720–727, 1999.
21. Cool CD, Rai PR, Yeager ME, Hernandez-Saavedra D, Serls AE, Bull TM, Geraci MW, Brown KK, Routes JM, Tuder RM, Voelkel NF. Expression of human herpesvirus 8 in primary pulmonary hypertension. N Engl J Med 349:1113–1122, 2003.
22. Miles SA, Rezai AR, Salazar-Gonzalez JF, Vander Meyden M, Stevens RH, Logan DM, Mitsuyasu RT, Taga T, Hirano T, Kishimoto T, Martinez-Maza O. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. Proc Natl Acad Sci USA 87:4068–4072, 1990.
23. An J, Sun Y, Sun R, Rettig MB. Kaposi's sarcoma-associated herpesvirus encoded vFLIP induces cellular IL-6 expression: the role of the NF-kB and JNK/AP1 pathways. Oncogene 22:3371–3385, 2003.