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Kaposi's sarcoma and human dermal microvascular endothelial cells infected with Kaposi's sarcoma-associated herpesvirus express CCL21

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Dear Sir,

Kaposi's sarcoma (KS) is the most common cancer in individuals with AIDS. Histologically, tumor cells form irregular, slit-like vascular structures. Numerous inflammatory cells are commonly present within KS tumors. In all clinical forms of KS, tumor cells and endothelial cells lining slit-like structures are infected with the KS-associated herpesvirus (KSHV) [1,2].

Tumor cells within KS express CD68 and CD34 as well as VEGFR-3, podoplanin, and LYVE-1. The last three are expressed by lymphatic endothelial cells [2]. CCL21 is usually expressed by lymphatic endothelial cells, and not blood vessel endothelial cells, as well, but its expression in KS has not been studied in detail. CCL21 is chemotactic for CCR7⁺ leukocytes, including lymph node (LN)-homing T cells and mature dendritic cells, and is critical for normal trafficking of these cells to LNs [3,4].

In this report, we show that CCL21 is expressed by round and spindle-shaped cells within KS tumors. We also show that KS tumors contain collections of CCR7⁺ cells, including inflammatory leukocytes. In addition, we demonstrate up-regulation of CCL21 in normal human dermal microvascular endothelial cells (HDMEC), which are putative precursor cells for KS spindle cells, following KSHV infection *in vitro*.

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The Institutional Review Boards at both Oregon Health & Science University and the VA Medical Center in Portland, Oregon approved the protocol. CCL21 and CCR7 expression was assessed in skin tissue obtained from healthy volunteers and from five patients with KS by immunohistochemistry. Five- μ m sections were incubated with either goat polyclonal anti-human 6Ckine IgG antibody (Ab) (R&D systems) or mouse monoclonal anti-human CCR7 IgM Ab (BD Biosciences) for 1 hr. Slides were then incubated with either biotinylated anti-goat IgG (Vector Laboratories) or biotinylated anti-mouse IgM (Vector Laboratories) for 30 min and Vectastain Elite ABC kit (Vector Laboratories).

HDMEC were immortalized by infection with the recombinant retrovirus LXSNI6 E6E7 derived from the amphotropic retrovirus-packaging cell line PA317 as previously described [5]. Immortalized HDMEC were infected with KSHV as previously described [5]. Briefly, BCBL-1 cells (AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases) were exposed to tetradecanoyl phorbol acetate (20 ng/mL) for 48 hr and cell-free KSHV inocula were obtained. Virus inocula were added to HDMEC for 4 hr in the presence of Polybrene (2 μ g/ml) followed by the addition of equal volumes of heparin-free culture medium for an additional 12 hr or overnight. For each experiment, mock infections were performed in parallel with BCBL-1 supernatants exposed to UV light to inactivate KSHV.

Quantitative real-time RT-PCR was performed as previously described [6] using RNA extracted from KSHV-infected or mock-infected HDMEC with Trizol® (Invitrogen). Primer sequences used were as follows: *CCL21* (sense), 5'-TGCTCCATCCCAGCTATCCT-3'; *CCL21* (anti-sense), 5'-GGTCTGCACATAGCTCTGCCT-3'; *LANA* (sense), 5'-CCAGGAAGTCCCACAGTGTTC-3'; *LANA* (anti-sense), 5'-GCCACCGGTAAAGTAGGACTAGAC-3'. Primers for GAPDH were purchased (Applied Biosystems).

Western blot was performed as previously described [7]. The Abs used in this study were goat polyclonal anti-human CCL21 Ab (R&D systems), mouse monoclonal anti-rabbit GAPDH Ab (Research Diagnostics Inc.), rabbit polyclonal anti-ERK1/2 Ab, and rabbit polyclonal anti-phosphorylated ERK1/2 Ab (Cell Signaling Technology).

As expected, some areas within dermis of normal skin and KS tumors showed small rings of CCL21 positive cells, which were also positive for podoplanin, a marker for lymphatic endothelial cells (Fig. 1a and data not shown). KS skin incubated with no primary antibody showed no stained cells (Fig. 1b). By contrast, many round and spindle-shaped cells within dermal tumors from all five patients with KS stained positive for CCL21 (Fig. 1c, d and data not shown). Infiltrating inflammatory leukocytes showed no evidence of CCL21 positivity (Fig. 1c, d and data not shown).

CCR7 is the only known receptor for CCL21 [3]. Spleen tissue, served as a positive control, showed CCR7⁺ cells as expected (Fig. 1e). KS tumors incubated with no primary antibody showed no stained cells (Fig. 1f). By contrast, we consistently found collections of CCR7⁺ cells, including inflammatory leukocytes scattered within KS tumors, suggesting that CCL21 expressed in KS lesions was functionally active (Fig. 1g, h and data not shown).

We next examined whether KSHV infection could up-regulate CCL21 expression within HDMEC. Using real-time RT-PCR, we first established high levels of infection in HDMEC by quantifying mRNA expression of KSHV *LANA*, a KSHV latent-cycle gene expressed in all KSHV-infected cells. As expected, in four separate experiments, *LANA* mRNA was expressed at levels 500-7,000 times higher than background levels measured in mock-infected HDMEC (Fig. 2a). In these same four mRNA samples, *CCL21* mRNA was expressed at levels 20-1,300 times levels detected in mock-infected HDMEC (Fig. 2b).

There was no correlation between *LANA* and *CCL21* expression, probably because of different regulation of these two genes. We also detected up-regulation of CCL21 protein in KSHV-infected endothelial cells by Western blotting (Fig. 2c).

CCL21 expression by HDMEC is increased by oncostatin M (OSM). We have previously shown that MAPK signaling is important in CCL21 expression of HDMEC induced by OSM [7]. KSHV infection of immortalized HDMEC up-regulates phosphorylation of Erk1/2 (Fig. 2d).

We postulate that CCL21 secreted by KS spindle cells may recruit CCR7⁺ cells into KS tumors rather than allow these cells to migrate to their normal destination (i.e., LNs). In this manner, KS tumors may act as “decoys” for LN-homing cells, which could in turn potentially lead to generation of abnormal immune responses to KSHV-infected cells. Further investigation is needed to explore this possible immune surveillance escape hypothesis.

KS is rich in cytokines and chemokines [8,9]. One of the cytokines found in abundance in KS is OSM [8]. OSM has been shown to act a potent mitogen for KS cells *in vitro* [9]. Interestingly, both OSM treatment and KSHV infection of HDMEC up-regulate CCL21 expression and phosphorylation of Erk1/2. It would be very interesting to examine whether MAPK inhibitors reduce CCL21 expression and phosphorylation of Erk1/2 in KSHV-infected HDMEC, as we previously showed in OSM-treated HDMEC [8]. The anecdotal case report of Kaposi’s sarcoma demonstrating complete clinical response after administration of sorafenib, an inhibitor of MAPK-mediated proliferation, encourages further investigation in this direction [10].

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References

1. Boshoff C, Schulz TF, Kennedy MM, Graham AK, Fisher C, Thomas A, et al. Kaposi’s sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nat Med.* 1995; 1:1274–8. [PubMed: 7489408]
2. Hong YK, Foreman K, Shin JW, Hirakawa S, Curry CL, Sage DR, Libermann T, et al. Lymphatic reprogramming of blood vascular endothelium by Kaposi sarcoma-associated herpesvirus. *Nat Genet.* 2004; 36:683–5. [PubMed: 15220917]
3. Yoshida R, Nagira M, Kitaura M, Imagawa N, Imai T, Yoshie O. Secondary lymphoid-tissue chemokine is a functional ligand for the CC chemokine receptor CCR7. *J Biol Chem.* 1998; 273:7118–22. [PubMed: 9507024]
4. Debes GF, Arnold CN, Young AJ, Krautwald S, Lipp M, Hay JB, et al. Chemokine receptor CCR7 required for T lymphocyte exit from peripheral tissues. *Nat Immunol.* 2005; 6:889–94. [PubMed: 16116468]
5. Moses AV, Fish KN, Ruhl R, Smith PP, Strussenberg JG, Zhu L, et al. Long-term infection and transformation of dermal microvascular endothelial cells by human herpesvirus 8. *J Virol.* 1999; 73:6892–902. [PubMed: 10400787]
6. Sugaya M, Watanabe T, Yang A, Starost MF, Kobayashi H, Atkins AM, et al. Lymphatic dysfunction in transgenic mice expressing KSHV k-cyclin under the control of the VEGFR-3 promoter. *Blood.* 2005; 105:2356–63. [PubMed: 15536152]
7. Sugaya M, Fang L, Cardones AR, Kakinuma T, Jaber SH, Blauvelt A, et al. Oncostatin M enhances CCL21 expression by microvascular endothelial cells and increases the efficiency of dendritic cell trafficking to lymph nodes. *J Immunol.* 2006; 177:7665–72. [PubMed: 17114436]

8. Ensoli B, Barillari G, Gallo RC. Cytokines and growth factors in the pathogenesis of AIDS-associated Kaposi's sarcoma. *Immunol Rev.* 1992; 127:147–55. [PubMed: 1506004]
9. Miles SA, Martínez-Maza O, Rezai A, Magpantay L, Kishimoto T, Nakamura S, et al. Oncostatin M as a potent mitogen for AIDS-Kaposi's sarcoma-derived cells. *Science.* 1992; 255:1432–4. [PubMed: 1542793]
10. Ardavanis A, Doufexis D, Kountourakis P, Rigatos G. A Kaposi's sarcoma complete clinical response after sorafenib administration. *Ann Oncol.* 2008; 19:1658–9. [PubMed: 18647963]

Fig. 1.

KS tumors contain CCL21⁺ spindle cells and CCR7⁺ cells (x 80). (a) CCL21⁺ dermal lymphatic endothelial cells (in brown) in normal skin. (b) Serial section of a KS specimen (also shown in c) incubated with no primary antibody showing no background staining. (c, d) Sections taken from two separate patients with KS showing spindle cells within dermal tumors staining for CCL21 (in brown). (e) CCR7⁺ positive cells in normal human spleen (in brown). (f) Serial section of a KS specimen (also shown in g) incubated with no primary antibody showing no background staining. (g, h) Sections taken from two separate patients with KS showing collections of infiltrating cells within dermal tumors staining for CCR7 (in brown).

Fig. 2.

KSHV infection of immortalized HDMEC induces CCL21 mRNA and protein expression and phosphorylation of Erk1/2. On four separate occasions, immortalized HDMEC were infected with KSHV or inactivated KSHV (i.e., mock-infection) and both (a) KSHV *LANA* and (b) *CCL21* mRNA expression were found to be markedly up-regulated compared to background signals using quantitative real-time RT-PCR. (c) CCL21 protein was also enhanced in KSHV-infected endothelial cells compared to mock-infected cells by Western blot analysis. GAPDH was used as a loading control. (d) KSHV infection of immortalized HDMEC up-regulates phosphorylation of Erk1/2, which is a key pathway for expression of CCL21 in these cells.