Int J Clin Exp Pathol 2016;9(5):5220-5229
www.ijcep.com /ISSN:1936-2625/IJCEP0022773

Original Article

Kaposi's sarcoma-associated herpesvirus infection induces overexpression of LYN in Kaposi's sarcoma

Fang Zhang[1], Xiaohua Tan[2], Ying Chen[3], Feng Guo[3], Yiming Xu[4], Zemin Pan[1], Yu Ji[1], Xianxian Ren[1], Lei Yang[3], Dongmei Li[1], Feng Li[1]

[1]School of Medicine, Shihezi University, Xinjiang 832000, China; [2]School of Medicine, Hangzhou Normal University, Hangzhou, Zhejiang 310036, China; [3]People's Hospital of Xinjiang Uygur Autonomous Region, Urumchi, Xinjiang 830001, China; [4]Hospital of Bayingolin Mongol Autonomous Prefecture, Korla, Xinjiang 841000, China

Received December 27, 2015; Accepted March 12, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: Kaposi's sarcoma-associated herpesvirus (KSHV) is an important pathogen for Kaposi's sarcoma (KS), a multiple hemangiosarcoma. We identified differentially expressed v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (*lyn*) gene in Xinjiang Uyghurs KS tumor tissues and normal skin tissues by Affymetrix microarray. Analyzed the influence of KSHV infection on the expression of LYN gene in human umbilical vein endothelial cells (HUVECs) to characterize the pathogenesis of KS and identify therapeutic targets for KS. According to the microarray and bioinformatics, LYN up-regulated in KS tissues primarily participated in response to biotic stimulus, immune response and signal transducer activity. Moreover, LYN was over-expressed in KS tissues which confirmed by immunohistochemistry (IHC) and real-time PCR. KSHV infection was detected in blood serum of 72 Xinjiang Uyghurs KS patients including the patients who provided the tissues to do microarray and 68 normal people by amplification of $KS330_{233}$. The seroprevalence of KSHV was 91.7% (66/72) in KS patients and 11.8% (14/68) in normal controls of Xinjiang province ($P<0.001$). To explore the relationship between LYN and KSHV, we extracted KSHV from BCBL-1 cells was used to infect primary cultured HUVECs. The levels of LYN in infected HUVECs and uninfected cells were evaluated by western blot. We noted that LYN was increased and a time-effect relationship with KSHV infection. Then infected cells were treated with a LYN inhibitor, PP2. PP2 inhibited LYN expression and decreased the cell viability ($P<0.01$) which was evaluated by western blot and Cell Counting Kit 8, respectively. Altogether the pathogenic mechanism of KSHV in KS may involve promotion of LYN overexpression in vascular endothelial cells.

Keywords: KSHV, KS, LYN, HUVECs, PP2

Introduction

Kaposi's sarcoma (KS) is an angiogenic, multifocal and inflammatory malignancy hemangiosarcoma. It was first described as a skin cancer affecting elderly patients by a Hungarian dermatologist named Moritz Kaposi in 1872 [1]. It consists of highly proliferative spindle-shaped cells that lead to the formation of abnormal vessels [2]. KS is also one of the most common cancers affecting patients with human immunodeficiency virus infection and acquired immune deficiency syndrome (HIV/AIDS).

Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV-8), is a gamma-2-herpesvirus that was identified in 1994 by Chang et al. from KS tissues using representational difference analysis (RDA) [3]. KSHV is widely implicated in the pathogenesis of Kaposi's sarcoma (KS) including AIDS-KS, classic KS, endemic KS, and iatrogenic KS, and is deemed to an important pathogen for KS. KSHV is also linked to lymphoproliferative malignancies including primary effusion lymphoma (PEL) and multicentric Castleman's disease (MCD) [4].

Epidemiological studies have revealed that the global seroprevalence of KSHV varies greatly. In African and Mediterranean regions, the seroprevalence of KSHV varies from 20% to 80%. In United States and Northern Europe, its seroprevalence is generally low [5]. In China, the prevalence of classic KS and AIDS-KS is high in the Xinjiang province. These cases are predomi-

nantly reported in the Uyghur and Kazakh ethnicities, and are rarely reported in the Han Chinese population [6]. KSHV seroprevalence is higher in areas of high KS incidence [7, 8]. The exact pathogenic mechanism of KSHV is unclear and warrants elucidation. However, the pathogenic effect of KSHV is known to involve the alteration of the expression and activity of host genes [9].

In this study, we wanted to found the differentially expressed genes in Xinjiang Uyghurs KS tissues by microarray. And the v-yes-1 Yamaguchi sarcoma viral related oncogene homolog (lyn; also named JTK8 and p53/56 LYN) gene was found up-regulated in Xinjiang KS tissues. LYN which encodes a non-receptor tyrosine kinase of the Src family is known to be closely related to cancer recently. Wheeler et al. have reported LYN-mediated cell motility and tumor growth in head and neck squamous cell carcinomas [10]. Hui Guan *et al.* found that inhibition of LYN decreases tumor growth and metastasis in Ewing's sarcoma [11]. Sutton *et al.* found that LYN regulates activation of epidermal growth factor receptors (EGFR) in lung adenocarcinoma cells through the PI3K/AKT signaling pathway which is an important pathway that can control cell proliferation and survival [12]. The Src kinase inhibitor PP2 is an inhibitor of LYN [13]. It is unclear whether KSHV infection induces up-regulation of LYN in vascular endothelial cells, and then promotes the proliferation and transformation to the spindle morphology.

To characterize the pathogenesis of KS, we investigated the differentially expressed genes in Xinjiang KS tissues and verified the up-regulation of LYN. We studied the relationship between KSHV infection and LYN expression. Then we used the LYN inhibitor, PP2, to treat KSHV-infected cells and then compared the variations in LYN and the cell viability.

Materials and methods

Sample collection

Tissue and serum samples of patients with KS were collected from Ili Friendship Hospital and the People's Hospital of Xinjiang Uygur Autonomous Region, Xinjiang, China. Clinical data for patients with KS were obtained by reviewing their medical records. None of these patients received chemotherapy or radiotherapy before the tissue samples were obtained. All histological diagnoses were confirmed by experienced pathologists in the hospital. The patient records and information were anonymized and de-identified prior to analysis. The human umbilical cord was collected from the First Affiliated Hospital of Medicine School, Shihezi University. Written informed consent from the donor was obtained for the use of this sample in research. Details of the investigation and the required informed consent have been examined and certified by the Ethics Committee of the First Affiliated Hospital of Medicine School, Shihezi University.

RNA extraction and Affymetrix array

Total RNA was isolated from skin biopsy specimens from healthy spots and lesion spots of patients with KS by using Trizol reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. The RNA concentration was measured using a Nano Drop 1000 system (Thermo Scientific, Waltham, MA, USA). Gene expression in 4 KS tumor tissues and normal skin tissues was analyzed using a Gene Chip Human Genome U133 Plus 2.0 Array (Affymetrix, Santa Clara, CA, USA). Production of biotinylated antisense complementary RNA (cRNA) and hybridization of the labeled cRNA to GeneChip were performed according to the manufacturer's protocols. After the microarray analysis, Mas 5 was used to normalize the data and screen for differentially expressed genes. The unpaired t-test *p*-value was required to be less than 0.05 to remove low-repeat differentially expressed genes. The fold change for upregulation or downregulation was defined as >2 or <0.5, respectively. Horizontal clustering and gene ontology (GO) were used to calculate the quantity of genes in the different nodes. Differentially expressed genes classified under Biological Process (BP) and Molecular Function (MF) was clustered.

Immunohistochemistry (IHC) and immunocytochemistry (ICC)

Sections (4 μm) were cut from the formalin-fixed paraffin-embedded tissue samples. The avidin-biotin-peroxidase protocol was used for IHC. Samples were incubated with antibodies against LYN (1:100, BD Biosciences, Franklin Lakes, NJ, USA) at 4°C overnight and visualized

Table 1. List of oligonucleotide primers

Target Gene	Primer ID	Sequence (5'-3')
KS330$_{233}$ first	Forward	TCCGTGTTGTCTACGTCCAG
	Reverse	AGCCGAAAGGATTCCACCAT
KS330$_{233}$ second	Forward	AATGACACATTGGTGGTATA
	Reverse	ACGGATTTGAC-CCCGTGTTC
lyn	Forward	GAACTCCCAGCAGGCTTACCAG
	Reverse	CATTCAGCGTATC CAATTCAGCA
β-actin	Forward	TTAGTTGCGTTACACCCTTTC
	Reverse	ACCTTCACCGTTCCAGTTT

using a DAB kit (Dako, Glostrup, DK). All sections were examined and scored by two pathologists in a blinded evaluation. Staining was scored based on intensity and proportion. The signal intensity was scored as 0, no staining; 1+, low intensity; 2+, moderate intensity; or 3+, high intensity. The extent of surface area containing the target protein was scored on a scale of 0-3 (0: no staining; 1+: present, but <20%; 2+: 20-50%; and 3+: >50%). The positivity score was calculated by multiplying staining intensity and surface area data by the tissue (range: 0-9), and the composite scores were separated using a four-tier system (negative: 0-1; 1+: 2-4; 2+: 5-7; and 3+: 8-9). We used CD34 (1:100, Dako) and CD31 (1:100, Dako) to validate the Human Umbilical Vein Endothelial Cells (HUVECs). The suspension containing HUVECs was placed on a glass slide for 12 hours. Cells were then immediately fixed with 4% paraformaldehyde (PFA) and incubated with primary antibodies at 4°C overnight. Fixed cells were visualized using a DAB kit (Dako).

PCR and real-time PCR

KSHV DNA was isolated from serum by using the SK1371UN I Q-10 Virus Gen Purification kit (Sangon Biotech Co., Ltd., Shanghai, China) following the manufacturer's instructions. Nested PCR was used to determine the specificity of the fragment $KS330_{233}$ of KSHV which is 233 bp. The sequences of first-round PCR primers and second-round primers are shown in **Table 1**. Primers, Taq polymerase, dNTP, and buffer were purchased from Dalian Treasure Biological Co., Ltd (Dalian, Liaoning, China). All samples were subjected to amplification by a T-gradient gradient PCR instrument (Biometra T3 Thermocycler, Gottingen, Germany). First-round amplification products were used as templates for the second round of amplification. PCR was initiated by 5 min incubation at 94°C, 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C and extension at 72°C for 45 s, and ended after a 5 min extension at 72°C. To eliminate false-negative and false-positive results, the $KS330_{233}$ fragment was used as a positive control template (provided by the Department of Dermatology, People's Hospital of Xinjiang Uygur Autonomous Region, China) and double-distilled water was used as a negative control. Real-time PCR was used to determine the expression of the differentially expressed gene *lyn* in KS tissues on an Applied Biosystems 7500 Real-Time PCR system (Foster City, CA, United States). The primers are presented in **Table 1**.

Cell culture

HUVECs were primary-cultured HUVECs from an umbilical cord sample collected from the First Affiliated Hospital of Medicine School, Shihezi University. The cord was inspected, and all areas with clamp marks were cut off and then perfused with 0.1% collagenase type II (Sigma, St Louis, MO, USA). The samples were digested for 15 min in a water bath at 37°C. Subsequently, the collagenase solution containing the endothelial cells was sedimented and resuspended in endothelial cell culture medium, and the endothelial cells were cultured in endothelial cell culture medium (Sciencell, Carlsbad, CA, USA) supplemented with 5% fetal bovine serum (FBS), 1% endothelial cell growth supplement (ECGS), and 1% penicillin/streptomycin solution (P/S).

Body cavity-based lymphoma cells (BCBL-1, KSHV+/EBV-) were originally purchased from ATCC (kindly provided by Dr. Dean Kedes, University of Virginia) and maintained in RPMI 1640 medium (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS. The two cell lines were maintained in a humidified atmosphere containing 5% CO_2 at 37°C.

Infection of HUVECs by KSHV

KSHV inoculum was obtained from BCBL-1 cells. The cells were induced with PMA for 72 h. Then, the medium containing PMA was removed and fresh medium was added to the cells, which were then cultured for 24 h. The collected BCBL-1 cells were placed in liquid nitrogen and then in water at 60°C. This procedure was repeated several times to disrupt the cell mem-

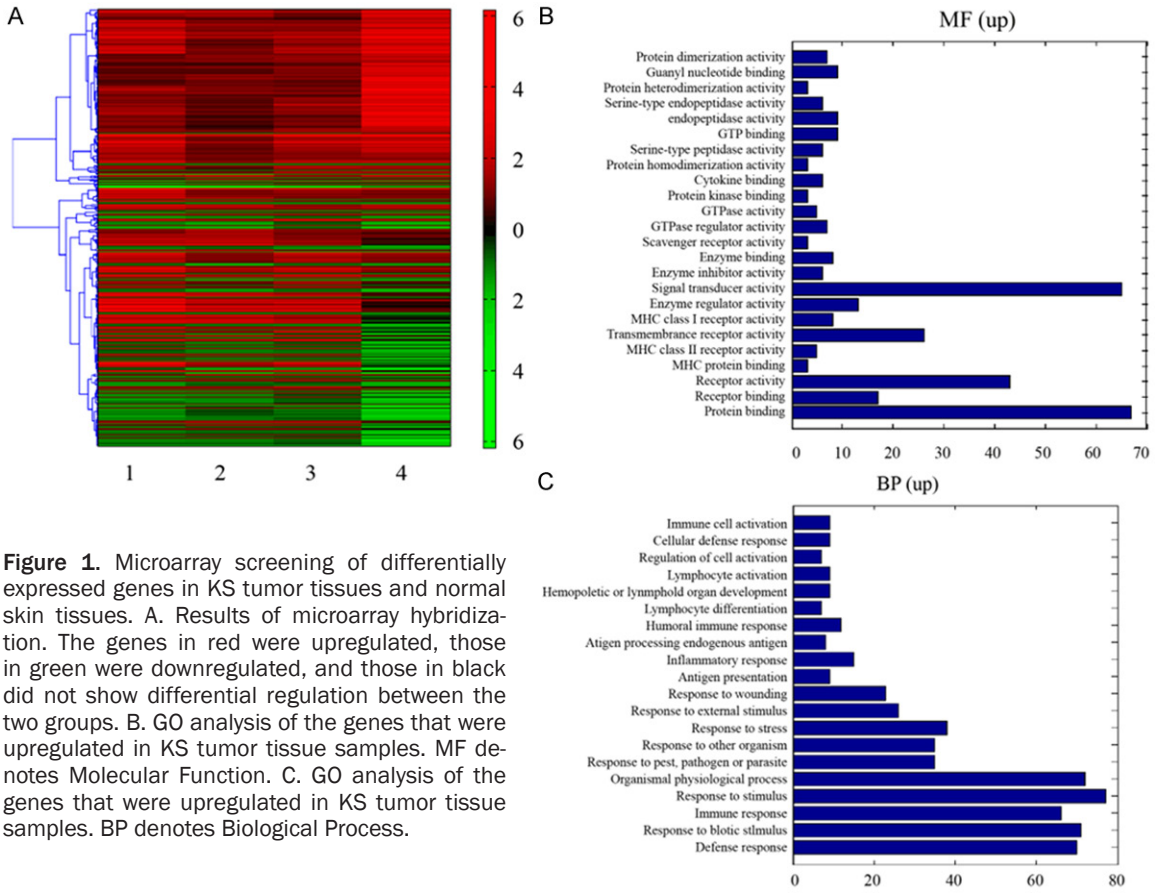

Figure 1. Microarray screening of differentially expressed genes in KS tumor tissues and normal skin tissues. A. Results of microarray hybridization. The genes in red were upregulated, those in green were downregulated, and those in black did not show differential regulation between the two groups. B. GO analysis of the genes that were upregulated in KS tumor tissue samples. MF denotes Molecular Function. C. GO analysis of the genes that were upregulated in KS tumor tissue samples. BP denotes Biological Process.

brane. The supernatant (containing KSHV) was obtained by centrifugation at 12,000 g for 30 min at 4°C. The virus inoculum (virus: ECM medium = 5:1) was used to infect HUVECs for 24 h, 36 h, 48 h, and 72 h.

Cell counting kit-8 (CCK-8) proliferation assay

CCK-8 was used to identify the effect of LYN inhibitor PP2 to treat HUVECs and BCBL-1 cells by evaluating cell viability. Cells were seeded in 96-well plates at a density of 3,000 cells per well and treated with the drugs in triplicate. CCK-8 (10 μl) was added to each well for the last 4 h of incubation and the absorbance at 450 nm was measured using an automated microplate reader. PP2 were dissolved in dimethyl sulfoxide (DMSO). Cells cultured in medium containing DMSO were used as the negative control. Then, we used PP2 to evaluate cell viability for 1 d, 2 d, 3 d, 4 d, and 5 d.

PP2-treated cells

Based on a previous study, we selected 10 μM PP2 to treat BCBL-1 cells and HUVECs. In the control group, the cells were treated with DMSO. Images of the cells were taken at 24 h, 48 h, and 72 h. After 72 h, we extracted the total protein for western blotting.

Western blot

Proteins were extracted from cells for western blotting. Cells were lysed on ice with pre-cooled cell lysis buffer for 20 min and the total protein was isolated. The protein lysate was electrophoresed on a 10% polyacrylamide gel and then transferred to PVDF membranes that were blocked in Tris-buffered saline with Tween 20 with 5% nonfat milk for 2 h. Then, the membranes were incubated overnight with the primary antibody at 4°C in TBST with 5% nonfat milk. Antibodies against Lyn (1:500 dilution; BD) and β-actin (1:1000 dilution; ZSGB-bio, Beijing, China) were used. Immunoreactive bands were visualized using horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence (ECL) detection (Millipore, Temecula, CA, USA).

Table 2. Up-regulated genes in Kaposi's sarcoma tumor tissues

Probe Set ID	Mean_ cont	Fold_ change	*t*-test	Represent	Gene Symbol	Gene Title
216920 s	427.5	2.790058	0.000382	M27331	TRGC2	T cell receptor gamma constant 2
226219_at	678.5	2.006635	0.001131	AW575123	ARHGAP30	Rho GTPase activating protein 30
202626_s_at	681.5	2.144167	0.001165	NM_002350	LYN	v-yes-1 Yamaguchi sarcoma viral related oncogene homolog
201641_at	958.25	3.747195	0.001213	NM_00335	BST2	Bone marrow stromal cell antigen2
1045_i_at	662.5	4.8447	0.001699	M21121	CCL5	Chemokine (C-C motif) ligand 5
239723_at	504.75	2.418029	0.00174	AA588092	SLC40A1	Solute carrier family 40 (iron-regulated transporter), member 1
231776_at	344.25	2.53159	0.002295	NM_005442.1	EOMES	Eomesodermin homolog (Xenopuslaevis)
1555691_a_at	515.25	2.026201	0.002535	NM_007360	KLRK1	Killer cell lectin-like receptor subfamily K, member 1
204661_at	593.25	4.276443	0.002621	NM_001803	CD52	CD52 molecule
214617_at	409.75	3.032337	0.002695	NM_005041	PRF1	Perforin 1 (pore forming protein)

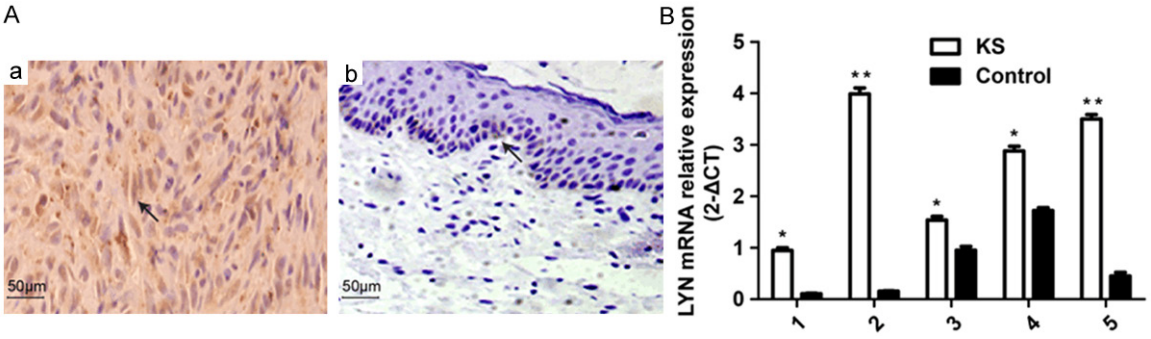

Figure 2. LYN expression in KS patients. A. Immunohistochemical analysis showed that expression of LYN increased in KS tumor tissues (200×). a. Staining of LYN in KS tumor tissues. b. Staining of LYN in normal skin tissue. B. Relative expression of LYN mRNA in KS, as determined by real-time PCR. **P*<0.05; ***P*<0.01.

Table 3. Results of the immunohistochemical analysis for LYN in tissue samples obtained from patients with Kaposi's sarcoma and normal controls

	Expression			
LYN	Positive (%)	Negative (%)	Total cases	*p*-value
KS	14/17 (82.4)	3/17 (17.6)	17	*P*<0.001
Normal	2/17 (11.8)	15/17 (88.2)	17	

Table 4. Seroprevalence of Kaposi's sarcoma-associated herpesvirus in patients with Kaposi's sarcoma and normal controls

KSHV	Positive (%)	Negative (%)	Total case	*p*-value
KS	66/72 (91.7)	6/72 (8.3)	72	*P*<0.001
Normal	14/68 (11.8)	54/68 (79.4)	68	

Statistical analysis

Statistical analysis were performed using Statistical Products and Services Solutions software (SPSS, version 17.0, Chicago, USA). The IHC results were analyzed by χ^2 test. The real-time PCR and the mean intensity as well as OD values were analyzed by t test. P<0.05 was considered statistically significant.

Results

Differentially expressed genes in KS tumor tissues and normal skin tissues were identified by microarray analysis

We isolated total RNA from 4 KS tumor tissue samples and adjacent normal skin tissue samples. In the heat map, red indicates upregulated genes, green indicates downregulated genes, and black indicates genes with intermediate expression (**Figure 1A**). In total, 246 up-regulated and 97 down-regulated genes were identified in KS (not shown). The top ten of 246 upregulated genes (**Table 2**) were selected by microarray analysis. LYN is one of the upregulated genes. LYN is a gene related with sarcoma oncogene. GO analysis classified the differentially expressed genes as associated with Molecular Function (MF) and Biological Process (BP). Based on the MF results (**Figure 1B**), the upregulated genes were primarily involved in

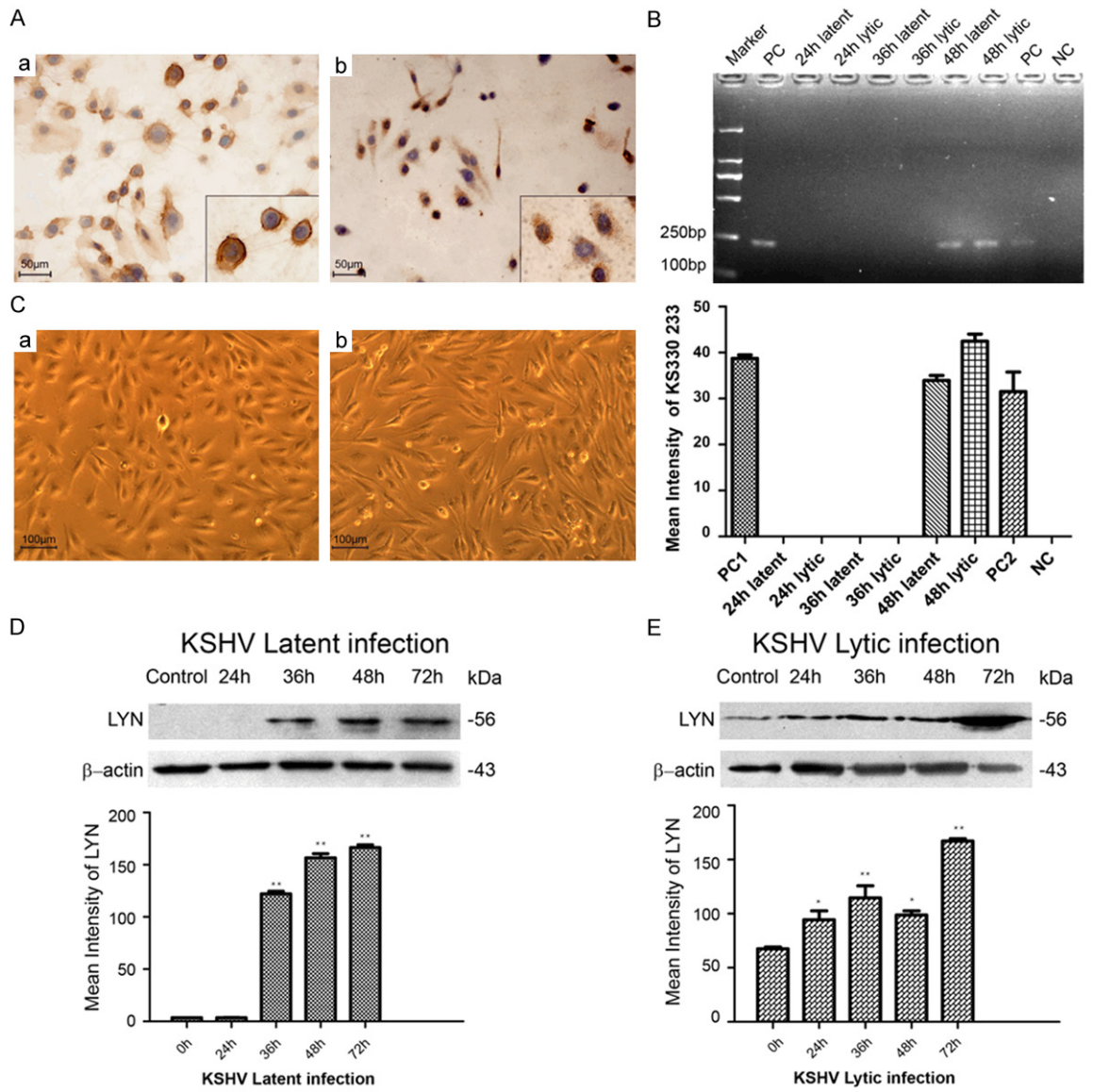

Figure 3. KSHV increased LYN expression in de novo infected HUVECs. A. Validation of HUVECs by immunocytochemistry. a. CD31 expression in HUVECs (200× and 400×). Cell membrane staining and cytoplasmic weak staining in HUVECs. b. CD34 expression in HUVECs (200× and 400×). Cytoplasmic staining in HUVECs. B. The effect of KSHV infection in HUVECs at different time points. Successful KSHV infection was observed after 48 h, with both lytic and latent infection states. PC: positive control; NC: negative control; latent: KSHV latent state; lytic: KSHV lytic state. C. Morphological changes in HUVECs de novo infected by KSHV (40×). a. Normal HUVEC morphology. b. HUVECs de novo infected by KSHV. After de novo infection with KSHV, HUVECs changed their morphology to long, spindle shaped cells. D. LYN expression at 24 h, 36 h, 48 h, and 72 h after latent KSHV infection. E. LYN expression at 24 h, 36 h, 48 h, and 72 h after lytic KSHV infection.

signal transducer activity, receptor activity, and protein-binding function. Based on the BP results (**Figure 1C**), the upregulated genes primarily participated in response to biotic stimulus, defense response and immune response. The function of LYN is consistent with the GO analysis. LYN often deregulated in cancers and linked to neoplastic transformation. The downregulated genes did not show any obvious functional classification (not shown).

Expression of LYN was increased in KS tumor tissue samples infected with KSHV

We collected 17 tissue samples from Xinjiang Uyghurs KS patients. LYN expression was found in 14 of 17 patients with KS (82.4%) (represen-

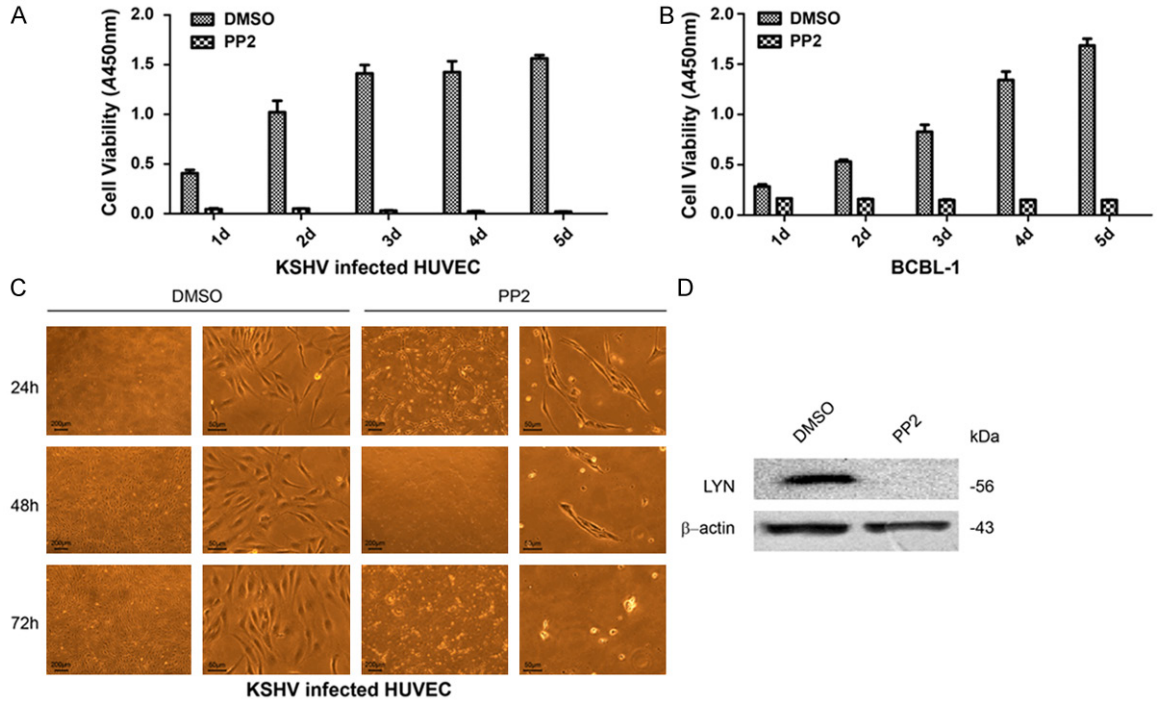

Figure 4. PP2 inhibited cell growth and LYN expression. A. Viability of KSHV-infected HUVECs after treatment with PP2 (10 μM). DMSO was added to KSHV-infected HUVECs and used as a control. B. Viability of BCBL-1 cells after treatment with PP2 (10 μM). DMSO was added to BCBL-1cells and used as a control. C. Microscopic examination of KSHV-infected HUVECs at 24 h, 48 h, and 72 h after PP2 treatment (10 μM). D. Western blot analysis of LYN in KSHV-infected HUVECs after PP2 treatment.

tative images shown in **Figure 2A**). IHC showed that LYN was over-expressed in KS tumor tissue samples ($P<0.001$) (**Table 3**). We also evaluated LYN mRNA expression in fresh tissues obtained from 5 Xinjiang Uyghurs patients with KS. We used the 2-ΔCT value to determine relative LYN mRNA expression (**Figure 2B**). KS tumor tissue samples showed higher LYN mRNA expression than samples collected from the control group ($P<0.05$).

Infection of KSHV was increased in blood serum of patients with KS

KSHV is widely acknowledged to be associated with the pathogenesis of KS; hence, we determined the seroprevalence of KSHV in Xinjiang Uyghurs patients with KS and in normal person. It included the patients who provided the tissues to do microarray. KSHV infection was found in 66 of 72 patients with KS (91.7%). Most KS patients were infected with KSHV ($P<0.001$, **Table 4**). It is worth noting that all of the KS patients to do microarray were infected by KSHV. Whether KSHV infection induces LYN overexpression needs to further study.

KSHV infection increased LYN expression in de novo infected HUVECs

KS is a multicentric angioproliferative neoplasm of endothelial origin. Hence, we used HUVECs that were de novo infected with KSHV as a cellular model. After primary cell culture, we verified the identity of the HUVECs based on morphological observations and ICC. HUVECs are short, spindle, or flat polygonal in shape; however, after culture for 4-5 days, the cells have paving stone morphology or show a spiral arrangement. ICC showed cell membrane staining or cytoplasmic weak staining for CD31 and cytoplasmic staining for CD34 in HUVECs (**Figure 3A**), which verifies that the cells were vascular endothelial cells.

After the primary culture was established, we used ECM medium containing KSHV viral filtrate to infect the HUVECs. The lytic-state KSHV is added PMA stimulate. To verify the effect of infection, we extracted the DNA of latent-state and lytic-state KSHV from BCBL-1 cells and infected HUVECs. We detected the specific fragment $KS330_{233}$ of KSHV to confirm the infection (**Figure 3B**) and found that KSHV suc-

cessfully infected HUVECs at 48 h. After de novo infection of HUVECs with KSHV, the cells changed their shape and showed a long, spindle morphology (**Figure 3C**). Then we extracted proteins from KSHV-infected HUVECs at 24 h, 36 h, 48 h, and 72 h. At 72 h, LYN expression peaked in HUVECs with latent and lytic KSHV infections (**Figure 3D**, **3E**).

Inhibition of LYN decreased the proliferation of KSHV-infected cells

PP2 is an inhibitor of LYN. We used PP2 (10 μM) to treat HUVECs infected by KSHV and the BCBL-1 cells. When LYN activity was inhibited, we found that cell proliferation significantly decreased ($P<0.01$) (**Figure 4A** and **4B**). We also observed HUVECs microscopically and found that proliferation of PP2-treated cells was significantly lower than that of untreated cells. The effect of PP2 on cell viability was time-dependent (**Figure 4C**). Moreover, after the addition of PP2, LYN expression was suppressed (**Figure 4D**).

Discussion

KSHV is one of the human oncogenic viruses. It is also a large double-stranded DNA virus. The length of KSHV DNA is 170 kb as determined by genome sequencing [14]. The hosts of KSHV are mainly endothelial cells and lymphocytes. When the host is infected by KSHV, this virus exhibits two phases: persistent latent infection and a transient lytic infection [15]. Treatment of the infected cells with phorbol myristate acetate (PMA) and histone deacetylase inhibitor (NaB) induces the virus to enter the lytic phase [16].

KSHV is the etiologic agent of Kaposi's sarcoma (KS). KS is an angiogenic, multifocal and inflammatory malignancy of endothelial cell and usually appears in skin tissues. Clinically, KS lesions have been described as patch, plaque and nodule. They consist of spindle cells, a proliferation of abnormal and leaky vessels and extravasated red blood cells with haemosiderin deposits. The most typical cell in KS lesions is these spindle-shaped cells. It's also known as KS cells. The infection of KSHV may be the primary cause to form these spindle cells.

The pathogenic mechanism of KSHV may involve alteration of the expression of host genes. We screened for differentially expressed genes in Xinjiang Uyghurs KS tumor tissues and normal skin tissues using microarray analysis and found up-regulated and down-regulated genes. According to the results of GO analysis, the up-regulated genes were primarily involved in signal transducer activity, receptor activity, and defense response. The down-regulated genes did not show obvious tendency. LYN was up-regulated in KS tumor tissues.

LYN is a member of the Src-family kinases (SFKs). Originally, the function of LYN was considered to regulate the B cell immune response. LYN kinase has been shown to be an important component in cytokine signal transduction in a variety of cell types and has been reported to play a key role in the growth and apoptotic regulation of hematopoietic cells [17], including stem cell factor (SCF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3) and interleukin-5 (IL-5). It is also a key molecule of the integrin signaling pathway and was known to play crucial roles in regulating cell proliferation, differentiation, migration, cell shape change and angiogenesis [18, 19]. In recent studies, LYN is known to be closely related to cancer. It was found to be overexpressed in many cancers, for example, Ewing's sarcoma [18], breast cancer [20], prostate cancer [21], and lung carcinoma [22]. Choi *et al.* [23] found that LYN induced the epithelial-mesenchymal transition (EMT) in breast cancer and that it is a target of dasatinib. The function of LYN conforms to the major function classification of GO analysis for the up-regulated genes in KS tumor tissues. And we verified LYN was overexpressed in KS tumor tissues at protein and mRNA levels. Because of Src family kinases playing critical roles in angiogenesis, LYN as a member of Src family kinases, it may mediate angiogenesis promoting tumor formation. We considered LYN is closely related with the pathogenesis of KS so we selected LYN for further study.

We also found all patients with KS that were evaluated in the microarray analysis were infected by KSHV. And 91.7% (66/72) of patients with KS in Xinjiang province of China were infected by KSHV. The high infection rate of KSHV and LYN overexpression may be related, as the high LYN expression may represent a response to KSHV infection.

We designed to investigate the relationship between LYN and KSHV. We extracted KSHV

from BCBL-1 cells that contained KSHV and then infected primary cultured HUVECs to simulate the process of viral infection. The expression of LYN increased over time with both latent and lytic KSHV infections. After KSHV infection, the morphology of the cells changed to a long, spindle shape and resembled that of KS cells. It is thus probable that KSHV infection causes LYN overexpression in KS patients. Inhibition of LYN by PP2 decreased the proliferation of KSHV-infected cells. Thus, LYN may serve as a potential therapeutic target in cancer.

Our study thus collectively showed that LYN overexpression is closely related with KSHV infection in KS patients and provides evidence to illustrate the pathogenic mechanism of KSHV. Furthermore, the study findings also provide a target for clinical treatment of KS, which is important for the patients in areas with a high KSHV infection rate.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Acknowledgements

This study was supported by Basic Research for Application of Xinjiang Province (No. 2015-AG015), Science and Technology bureau of Xinjiang Production and Construction Corps), and Research Project of High Level Talents (No. RCZX201332, Shihezi University).

Disclosure of conflict of interest

None.

Address correspondence to: Drs. Dongmei Li and Feng Li, School of Medicine, Shihezi University, Xinjiang 832000, China. Tel: 86-993-2057882; Fax: 86-993-2057882; E-mail: lidong_abc@126.com (DML); Tel: 86-993-2057136; E-mail: lifeng7855@126.com (FL)

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