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Notch Signal Transduction Induces a Novel Profile of Kaposi's Sarcoma-Associated Herpesvirus Gene Expression

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Kaposi's sarcoma-associated herpesvirus (KSHV) RTA transcription factor is recruited to its responsive elements through interaction with RBP-Jκ that is a downstream transcription factor of the Notch signaling pathway that is important in development and cell fate determination. This suggests that KSHV RTA mimics cellular Notch signal transduction to activate viral lytic gene expression. Here, I demonstrated that unlike other B lymphoma cells, KSHV-infected primary effusion lymphoma BCBL1 cells displayed the constitutive activation of ligand-mediated Notch signal transduction, evidenced by the Jagged ligand expression and the complete proteolytic process of Notch receptor I. In order to investigate the effect of Notch signal transduction on KSHV gene expression, human Notch intracellular (hNIC) domain that constitutively activates RBP-Jκ transcription factor activity was expressed in BCBL1 cells, TRExBCBL1-hNIC, in a tetracycline inducible manner. Gene expression profiling showed that like RTA, hNIC robustly induced expression of a number of viral genes including K5 immune modulatory gene resulting in downregulation of MHC I and CD54 surface expression. Finally, the genetic analysis of KSHV genome demonstrated that the hNIC-mediated expression of K5 during viral latency consequently conferred the downregulation of MHC I and CD54 surface expression. These results indicate that cellular Notch signal transduction provides a novel expression profiling of KSHV immune deregulatory gene that consequently confers the escape of host immune surveillance during viral latency.

Keywords: KSHV, Notch, K5, MHC I, CD54

Kaposi's sarcoma-associated herpesvirus (KSHV), also called human herpesvirus 8 (HHV8), is thought to be an etiologic agent of Kaposi sarcoma (KS) (Chang *et al.*, 1994). KSHV is also associated with two diseases of B cell origin: primary effusion lymphoma and an immunoblast variant of Castleman disease (Boshoff *et al.*, 1995; Cesarman *et al.*, 1995). DNA sequence analysis of the KSHV genome revealed a number of cellular homologs (Russo *et al.*, 1996; Neipel *et al.*, 1997; Nicholas *et al.*, 1997). Among those, K3 and K5 exhibit 40% amino acid identity to each other (Russo *et al.*, 1996; Nicholas *et al.*, 1997) and are expressed at the early lytic cycle of viral replication (Nicholas *et al.*, 1997; Sun *et al.*, 1999). Both the K3 and K5 gene products are related to the immediate-early gene (IE1) product of bovine herpesvirus 4 and the ORF12 of HVS (Nicholas *et al.*, 1997). All of these open reading frames contain two C_4HC_3 zinc finger motifs at the amino terminus, a hydrophobic transmembrane region in the central region but are of varying size in the carboxyl terminal tail (Nicholas *et al.*, 1997). Interestingly, K3 and K5 have been shown to downregulate the surface expression of MHC class I and CD54 (ICAM1) surface molecules with different activity and specificity. Biochemical analyses demonstrate that downregulation of class I molecules induced by K3 and K5 is likely due to their rapid endocytosis. K3- and K5-mediated downregulation of MHC I and CD54 suppresses the activation of cytotoxic T cells and natural killer cells, which ultimately protects KSHV-infected cells from the destruction by immune effector cells (Ishido *et al.*, 2000).

An important step in the herpesvirus life cycle is the switch from latency to lytic replication. The KSHV replication and transcription activator (RTA)

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plays a central role in this switch. Ectopic expression of KSHV RTA is sufficient to disrupt viral latency and activate lytic replication to completion (Lukac *et al.*, 1998; Sun *et al.*, 1998; Gradoville *et al.*, 2000; Song *et al.*, 2002). RTA activates the expression of numerous viral genes in the KSHV lytic cycle, including its own promoter, polyadenylated nuclear RNA, K12, ORF57, vOX-2, viral G protein-coupled receptor (K14/vGPCR), and vIRF1. KSHV RTA has been shown to be recruited to their responsive elements through interaction with the transcription factor RBP-Jκ (Hsieh and Hayward 1995; Ling and Hayward 1995; Liang *et al.*, 2002). RBP-Jκ binding sites are present in a number of RTA-regulated viral promoters. RBP-Jκ, which was originally purified and characterized by Kawaichi *et al*. (1992) and Hamaguchi *et al*. (1992), is highly conserved in evolution from nematodes to humans. Biochemical and genetic studies have demonstrated that RBP-Jκ acts downstream of the receptor Notch. Activation of the Notch receptor by binding of its ligands Delta, Jagged, or Serrate leads to proteolytic cleavage of the receptor at the inner side of the membrane (Radtke and Raj, 2003). The Notch intracellular domain (NIC) is then translocated to the nucleus, where it activates genes by interacting with RBP-Jκ. RTA may thus be regarded as functional homologs or mimickers of the activated Notch protein. Indeed, RBP-Jκ has been shown to be a critical component in mediating RTA activation of several KSHV target genes, including those for ORF57, thymidine kinase, and K14/vGPCR (Liang *et al.*, 2002; Liang and Ganem, 2004). Furthermore, RBP-Jκ plays an essential role in RTA-mediated lytic reactivation of KSHV, since such reactivation is completely defective in RBP-Jκ -/- cells while being efficient in RBP-Jκ +/+ cells (Liang and Ganem, 2003). This indicates that RBP-Jκ plays an important role in the RTA-mediated lytic gene expression by directing RTA to viral target promoters through interaction.

The great puzzle of KSHV pathogenesis is that certain viral lytic genes either behave as oncogenes or have immune modulatory properties (Hayward, 2003). However, either these activities may be alleviated during the lytic replication cycle because cell growth is blocked by RTA-induced lytic replication. In the other hand, it is possible that cellular signal transduction, for example Notch signal pathway, may induce a unique expression profile of KSHV growth and immune deregulatory genes that is distinct from that of latency program as well as lytic program. To test this hypothesis, I constructed KSHV-infected primary effusion lymphoma BCBL1 cells (TRExBCBL1-hNIC) expressing the constitutively active form of human Notch intracellular (hNIC) domain in a tetracycline inducible manner. It showed that hNIC specifically induced the expression of a number of KSHV genes including K5. Particularly, hNIC-mediated activation of K5 gene expression conferred the downregulation of MHC I and CD54 surface expression. These results demonstrate that cellular hNIC induces the expression of a selected subset of KSHV genes, in particular immune deregulatory gene, independently of RTA. Thus, cellular Notch signal transduction provides a novel viral gene expression profile during latency.

Materials and Methods

Cell culture

To generate TRExBCBL1-hNIC and TRExBCBL1-RTA cells, BCBL1 cells persistently infected by KSHV were engineered to establish a Flp-In/TetR-inducible system. This system generated stable, tetracycline-inducible mammalian expression cell lines by taking advantage of a yeast DNA recombination system which uses Flp recombinase and site-specific recombination to facilitate integration of the genes of hNIC or RTA (Nakamura *et al.*, 2003). 293T, TRExBCBL1-hNIC and TRExBCBL1-RTA cells were grown in Dulbecco's modified Eagle's medium or RPMI 1640 supplemented with 10% fetal calf serum.

Immunoblot assay

Cells were harvested and resuspended in lysis buffer (150 mM NaCl, 0.5% Nonidet P-40, and 50 mM HEPES buffer [pH 8.0]) containing protease inhibitors at 0 or 48 h Doxycyclin treatment. After centrifugation, supernatant which was contained polypeptides from 2 $\times 10^6$ cells were resolved by SDS-PAGE and transferred to nitrocellulose membrane. Immunoblot detection was performed with 1:5 or 1:5000 dilutions of antibodies with the enhanced chemiluminescence system (Amersham, USA).

Real-time, quantitative PCR for KSHV

RNA was isolated as previously described (Chang *et al.*, 2005) using RNAzol (Tel-Test, USA). Poly A-mRNA was prepared using dT-beads (Qiagen Inc, USA) and reverse-transcribed using Superscript-II reverse transcriptase (Life Technologies, MA) according to the manufacturer's recommendations. The procedures for real-time QPCR analysis have been previously described (Staudt *et al.,* 2004; Papin *et al.,* 2005). The final PCR reaction contained 2.5 μl primer mix (final concentration 166 nM), 7.5 μl 2 × SYBR PCR mix (Applied Biosystems, USA), and 5 μl sample. To guard against contamination and handling errors, all real-time QPCR reactions were assembled in a segregated clean room using filtered pipette tips and a robot. Real-time PCR was performed using an ABI

PRIZM 5700 machine (Applied Biosystems, USA) and universal cycle conditions.

Flow cytometry

Cells (5×10^5) were washed with RPMI medium containing 10% fetal calf serum and incubated with primary and secondary antibodies for 30 min at 4°C (Pharmingen, USA). After washing, each sample was fixed with 4% paraformaldehyde, and FACS analysis was performed with a FACScan (Becton Dickinson, USA).

Generation of a recombinant KSHV mutant BAC36-DK5

We used the approach of random transposon-based mutagenesis to generate KSHV mutants as previously described (Zhou *et al*., 2002; Ye *et al.*, 2004). The EZ:TN <KAN-2> insertion kit (Epicentre, USA), originally developed from the hyperactive Tn*5* transposition system, is highly efficient in randomly inserting its specific primer binding sites and a kanamycin-resistance (Kan^r) cassette into the target DNA *in vitro*. Briefly, purified recombinant KSHV BAC36 DNA was reacted with the transposon cassette (transposon and Kan^r) DNA in the presence of Tn*5* transposase for 2 h. An aliquot of the reaction mixture was then used to transform *Escherichia coli* strain DH10B by electroporation, and bacterial clones carrying the transposon cassette were selected on Luria-Bertani agar plates in the presence of 25 μg of kanamycin/ml. To identify the insertion point, BAC DNA purified from each of the kanamycin-resistant clones was subjected to DNA sequencing with two primers (P1 and P2) that are located at both ends of the transposon cassette. One of the clones, designated BAC36-DK5, with a transposon cassette inserted at genomic position 26388 [based on KSHV sequence U75698 (Russo, *et al.*, 1996)] near the N-terminal domain of K5, was selected for further characterization in this study.

Results

Constitutive activation of Notch signal transduction in KSHV-infected PEL cells

Analysis of viral mRNA expression both *in vivo* within Kaposi's sarcoma lesions and *in vitro* in infected PEL and endothelial cells indicates that KSHV gene expression is restricted to a small subset of latent genes. To test whether Notch signaling affected KSHV gene expression during latent program, the intracellular cytoplasmic region of human Notch receptor (hNIC), which induces the constitutive activation of RBP-Jκ-mediated transcription, was expressed in KSHV-infected BCBL1 cells in a tetracycline inducible manner. These cells were designated as TRExBCBL1-hNIC. TRExBCBL1-cDNA5 cells were generated as parental cells. TRExBCBL1-cDNA5 cells and TRExBCBL1-hNIC cells were treated with 1 μg/ml of Doxy for 0, 3, 6, 12, 24, 48, and 72 h to turn on human Notch receptor (hNIC). Cells were harvested and prepared protein from lysates. hNIC expression was detected by Flag antibody (1:5000). Tubulin expression was used as a loading control. Immunoblot assay showed that treatment of these cells with Doxy rapidly induced hNIC expression (Fig. 1A). This indicated that TRExBCBL1-hNIC cells were proper system to check Notch signaling affected KSHV gene expression.

Prior to investigating hNIC effect on KSHV gene expression, we examined the level of endogenous Notch signaling activity. KSHV-infected TRExBCBL1 -vector, TRExBCBL1-hNIC and TRExBCBL1-RTA cells, and virus-free TRExBJAB-vector, TRExBJAB-hNIC and TRExBJAB-RTA cells were Doxy-induced

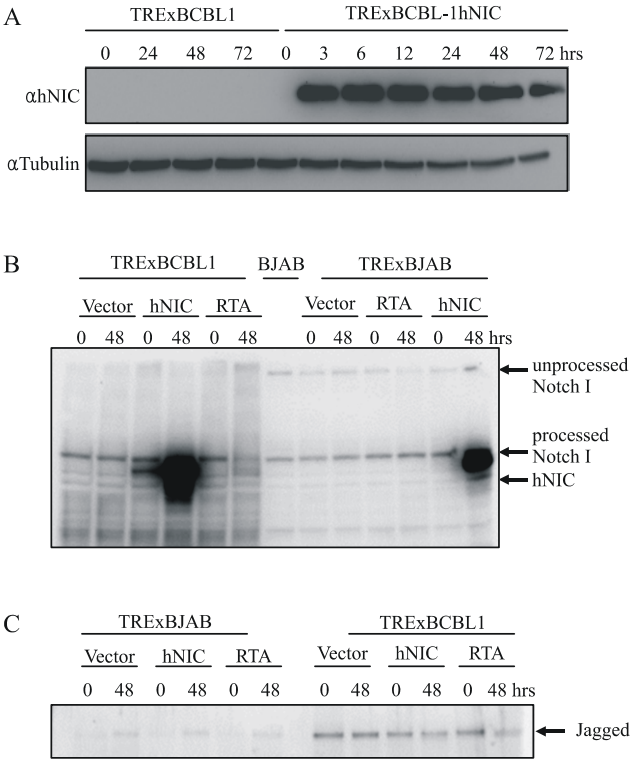

Fig. 1. Constitutive activation of Notch signal transduction in KSHV-infected PEL cells. (A) Induction of hNIC expression TRExBCBL1 and TRExBCBL1-hNIC cells were stimulated with 1 μg/ml Doxy for 0, 24, 48 or 72 h. Total cell lysates were used for immunoblotting with anti-Flag and anti-tubulin antibodies. Notch receptor I processing (B) and Jagged expression (C). TRExBJAB-Vector, TRExBJAB-hNIC, TRExBJAB-RTA, TRExBCBL1 -Vector, TRExBCBL1-hNIC, and TRExBCBL1-RTA cells were stimulated with 1 μg/ml Doxy for 0 and 48 h. Total cell lysates were used for immunoblotting with anti-Notch receptor I and anti-Jagged antibodies.

for 48 h and used for immunoblotting with anti-Notch receptor I and anti-Jagged ligand antibodies. While Notch receptor I underwent partial proteolytic processing in virus-free TRExBJAB cells, it showed near complete proteolytic processing in TRExBCBL1 cells (Fig. 1B). Furthermore, Jagged was constitutively expressed in TRExBCBL1 cells, but not in TRExBJAB cells (Fig. 1C). These results indicated that KSHV-infected BCBL1 cells displayed the constitutive activation of ligand-mediated Notch signal transduction, evidenced by the Jagged expression and the complete proteolytic process of Notch receptor I.

Genome-wide effects of hNIC on KSHV gene expression

In order to evaluate the impact of hNIC on the KSHV life cycle, we analyzed whether hNIC expression affected KSHV gene expression in latently infected BCBL1 cells. Approximately 1×10^7 TRExBCBL1-vector cells and TRExBCBL1-hNIC cells were treated with Doxy for 0, 24, and 48 h and mRNAs were isolated, reverse transcribed, and analyzed using the previously developed real-time quantitative reverse transcription-PCR (RT-QPCR) array for KSHV. Hierarchical clustering was performed with KSHV genes only, not on the samples, which were time series. Clustering was based on standard correlation metric. It showed that hNIC expression efficiently induced expression of a subset of KSHV genes (Table 1). hNIC-induced KSHV genes were divided into 5 subgroups according to their time frame expression profile (Table 1). Most of the KSHV genes (57%), designated as Group I, were rarely affected by hNIC. Group II genes showed low level of expression before Doxy treatment, whereas their expressions were rapidly induced upon Doxy treatment (Table 1 and 2). Expression of Group III genes, for instance, ORF53 and ORF 55, was detected after 12 h of Doxy treatment. Group IV consisted of 29% of KSHV genes that were induced after 24 h of Doxy treatment (Table 1 and 2). Finally, expression of group V genes including ORF18, ORF24, ORF27, ORF31, ORF62, ORF68, K9, and K10 were detected after 48 h of

Table 1. KSHV gene expression profile induced by hNIC

	0 h	12 h	24 h	48 h		0 h	12 h	24 h	48 h
K5 (E)	+	+	+	+	ORF66 (E)	−	−	+	+
ORF11 (UK)	+	+	+	+	ORF70 (UK)	−	−	+	+
K7 (UK)	+	+	+	+	ORF72 (L)	−	−	+	+
ORF53 (L)	−	+	+	+	K6 (E)	−	−	+	+
ORF55 (L)	−	+	+	+	K2 (E)	−	−	+	+
ORF4 (UK)	−	−	+	+	ORF17 (E)	−	−	+	+
K3 (UK)	−	−	+	+	ORF35 (UK)	−	−	+	+
vIRF3 (L)	−	−	+	+	ORF75 (UK)	−	−	+	+
ORF16 (E)	−	−	+	+	K4 (E)	−	−	+	+
ORF23 (UK)	−	−	+	+	ORF37 (E)	−	−	+	+
ORF30 (L)	−	−	+	+	ORF39 (L)	−	−	+	+
ORF32 (L)	−	−	+	+	ORF49 (E)	−	−	+	+
ORF53 (L)	−	−	+	+	ORF18 (UK)	−	−	−	+
ORF38 (UK)	−	−	+	+	ORF24 (UK)	−	−	−	+
ORF40 (E)	−	−	+	+	ORF27 (L)	−	−	−	+
ORF44 (E)	−	−	+	+	ORF31 (E)	−	−	−	+
ORF46 (E)	−	−	+	+	ORF62 (L)	−	−	−	+
ORF48 (IE)	−	−	+	+	ORF68 (E)	−	−	−	+
ORF52 (L)	−	−	+	+	K9 (E)	−	−	−	+
ORF57 (E)	−	−	+	+	K10 (UK)	−	−	−	+

(L), Latent gene; (IE), Immediate Early gene; (E), Early gene; (UK), Unknown gene

Table 2. KSHV gene groups induced by hNIC

	Group I	Group II	Group III	Group IV	Group V
Time of Gene Expression	No	0 h	12 h	24 h	48 h
% of Expressed KSHV gene	57% (53/93)	3.2% (3/93)	2.2% (2/93)	29% (27/93)	8.6% (8/93)

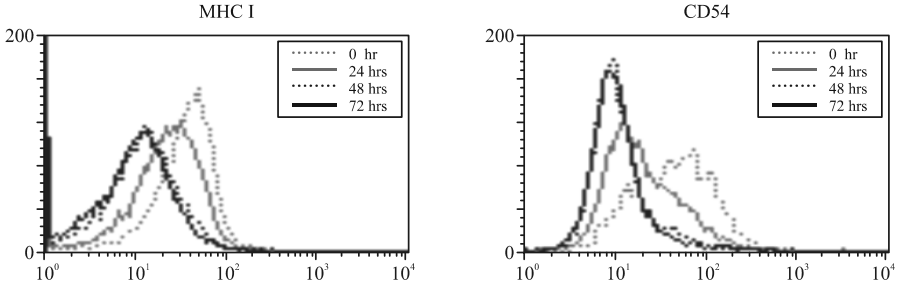

Fig. 2. MHC I and CD54 surface expression. TRExBCBL1-hNIC cells were treated with 1 μg/ml Doxy for 0, 24, 48, and 72 h, fixed, and then reacted with antibodies indicated at the upper side of panel for flow cytometry. Gray (-----) colored line, 0 h; gray (——) colored line, 24 h; black (-----) colored line, 48 h; and black (——) colored line, 72 h.

Doxy treatment. These results showed that like RTA, Notch signaling induced expression of a selected subset of KSHV genes. However, unlike RTA, hNIC was not capable of evoking the full repertoire of lytic viral gene expression.

Enhancement of K5-mediated downregulation of lymphocyte surface antigens by hNIC

Since hNIC induced K5 expression, we examined the effect of hNIC on lymphocyte surface antigen expression. TRExBCBL1-vector and TRExBCBL1-hNIC cells were treated with Doxy for 0, 24, 48, and 72 h, and tested for expression of various surface antigens including MHC I and CD54. It showed that hNIC expression induced the strong downregulation of MHC I and CD54 on TRExBCBL1-hNIC cells (Fig. 2).

To further delineate the effect of hNIC on the K5-mediated downregulation of lymphocyte surface antigens in the context of viral genome, we employed recombinant KSHV Bacmid genetics. Using recombinant KSHV BAC36 as a template, we applied random transposon-based mutagenesis to generate recombinant mutants with disruptions in the genes of interest. DNA sequencing of integration junctions identified one of the mutants as having an insertion of the transposon cassette into the N-terminal domain of K5 at genomic position 26,388, which was located 95 bp downstream of the translation start codon ATG of K5 (Fig. 3A). The insertion would disrupt the proper expression and function of K5 protein. We named this KSHV mutant BAC36-DK5.

293A cells were transfected with wt KSHV BAC36 or mutant KSHV BAC36-DK5 and selected for hygromycin resistance, followed by sorting GFP-positive cells. Hygromycin-resistant, GFP-positive 293A/KSHV BAC36 cells or 293A/KSHV BAC36-DK5 cells were examined for K3, K5, and RTA expression by immunoblotting analysis with their specific antibodies. It showed that K5 protein was detected in 293A/KSHV BAC36 cells, but not in 293A/KSHV BAC36-DK5 cells (Fig. 3B). In contrast, RTA and K3 expression was not observed at any detectable level in both cells (data not shown).

293A/KSHV BAC36 cells and 293A/KSHV BAC36-DK5 cells were then compared to untransfected 293A cells for the surface expression of MHC I and CD54. It showed that 293A/KSHV BAC36 cells showed detectable reductions of MHC I and CD54 surface expression compared to untransfected 293A cells, whereas 293A/KSHV BAC36-DK5 cells showed no alteration in MHC I and CD54 surface expression (Fig. 3C). This indicated that downregulation of MHC I and CD54 surface expression was strongly correlated with the K5 expression in 293A/KSHV BAC36 cells. To further demonstrate the effect of hNIC on K5 expression, 293A, 293A/KSHV BAC36, and 293A/KSHV BAC36-DK5 cells were transfected with vector or hNIC and then examined for K3, K5, and LANA expression. It showed that hNIC slightly induced K5 expression in 293A/KSHV BAC36 cells but not in 293A/KSHV BAC36-DK5 cells, whereas it did not affect K3 and LANA expression in both 293A/KSHV BAC36 and 293A/KSHV BAC36-DK5 cells (Fig. 3B and data not shown). 293A, 293A/KSHV BAC36, and 293A/KSHV BAC36-DK5 cells

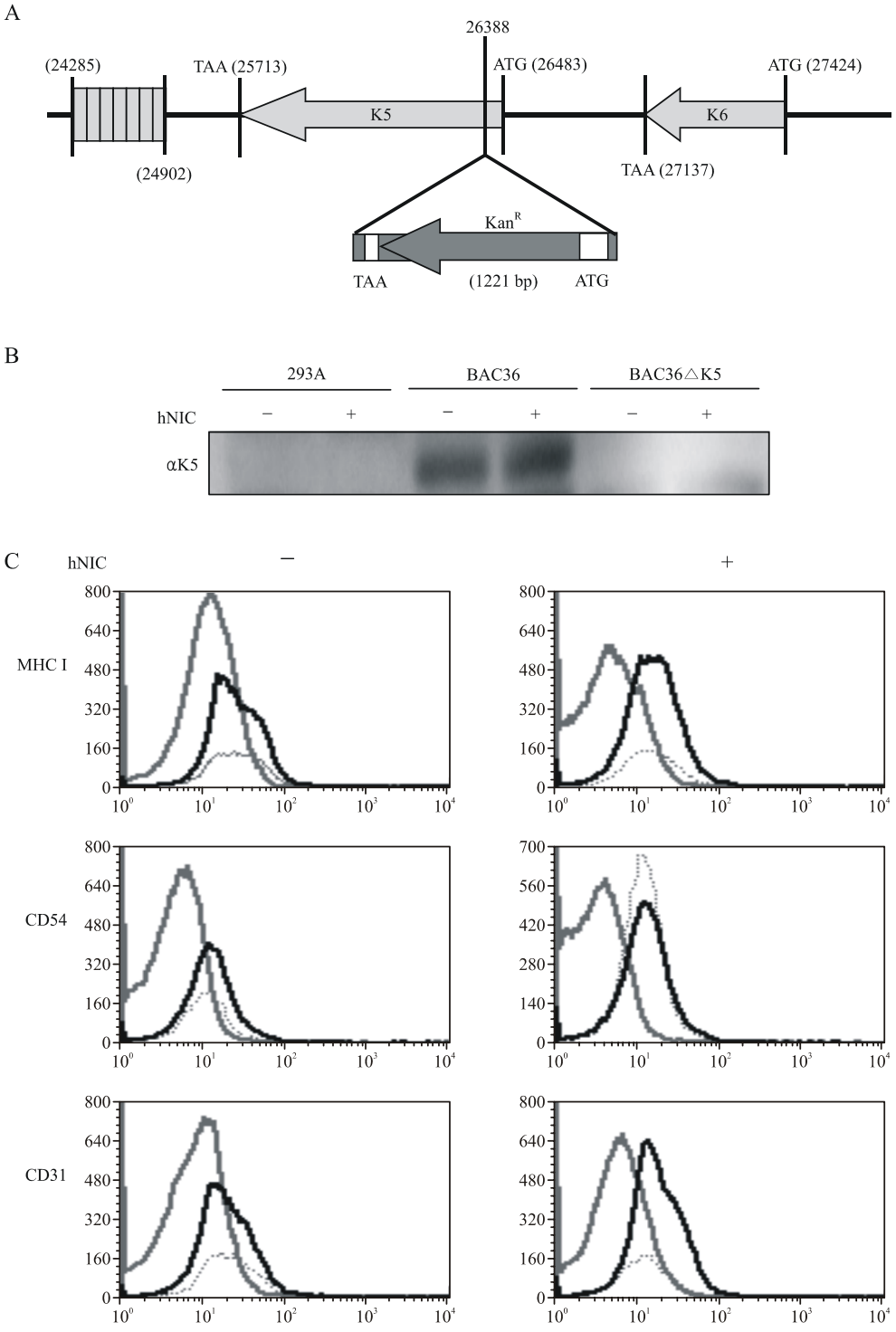

Fig. 3. hNIC induces the K5-mediated downregulation of lymphocyte surface antigens. (A) Schematic illustration of transposon-based mutagenesis for generating KSHV mutant BAC36-DK5. (B) Immunoblot analysis. 293A, 293A/KSHV BAC36, and 293A/KSHV BAC36-DK5 cells were transfected with vector or hNIC. At 48 h post-transfection, whole cell lysates were used for immunoblotting with anti-K5 antibody. (C) Flow cytometry analysis. 293A, 293A/KSHV BAC36, and 293A/KSHV BAC36-DK5 cells were transfected with vector or hNIC. At 48 h post-transfection, the surface expression of MHC I, CD54 and CD31 was examined by flow cytometry analysis. Gray (-----) colored line, 293A cells; gray (——) colored line, 293A/KSHV BAC36; and black (——) colored line, 293A/KSHV BAC36-DK5.

transfected with hNIC expression vector were also examined for MHC I and CD54 surface expression by flow cytometry. It showed that hNIC expression enhanced the downregulation of MHC I and CD54 surface expression on 293A/KSHV BAC36, but not on 293A and 293A/KSHV BAC36-DK5 cells (Fig. 3C). These results indicated that the K5 expression in latently infected 293A cells resulted in the downregulation of MHC I and CD54 surface expression and that the increase of K5 expression induced by hNIC further enhanced the downregulation of these lymphocyte surface antigens.

Discussion

The previous report (Liang *et al.*, 2002) has shown that the KSHV RTA protein mimics cellular Notch signal transduction by interacting with RBP-Jκ and by activating RBP-Jκ-dependent activation of viral lytic gene expression. Here, I also demonstrate that the constitutively active form of human Notch signaling molecule, hNIC, is partially exchangeable with RTA in viral lytic gene expression. Microarray data showed that hNIC robustly induced expression of a number of viral genes including K5. Despite the broad effect of hNIC on KSHV gene expression, hNIC was not capable of inducing the complete cycle of viral lytic replication, suggesting that a number of other cellular partners are required for RTA to evoke the full repertoire of lytic viral gene expression and thereby lytic replication. Interestingly, K5 and ORF11 genes showed the faster kinetics of expression upon hNIC expression compared to the rest of other viral genes, suggesting that they may be the initial targets for Notch signal transduction. In fact, we have recently identified the potential six RBP-Jκ binding sites in the K5 promoter region (Chang *et al.*, 2005). This indicates that Notch signal transduction is an important cellular pathway to regulate K5 gene expression.

Tomescu *et al.* (2003) has described the surface downregulation of MHC I, CD31, and CD54 immunoregulatory proteins by KSHV in newly infected endothelial cells. While KSHV lytic protein K3 and K5 are capable of downregulating MHC I and/or CD54, their contribution to immune evasion by KSHV during latency is less clear. This study showed that Notch signal transduction induced K5 expression independent of RTA. Thus, it is possible that K5 may express at a low level in latently infected cells, which is sufficient to induce surface molecule downregulation. In addition, under this condition, K5 expression is likely independent of the lytic transcription factor RTA, but dependent on the Notch signal-mediated RBP-Jκ transcription factor. In fact, I also showed that KSHV-infected BCBL1 cells likely displayed the constitutive activation of ligand-mediated Notch signal transduction, evidenced by the Jagged expression and the complete proteolytic process of Notch receptors. Furthermore, a recent report has shown that KSHV infected cells have not only elevated level of activated Notch and but also activated level of Notch-mediated transcription activity (Curry *et al.*, 2005)**.** Furthermore, inhibitors, which block Notch activation, resulted in apoptosis in primary and immortalized KS cells. The results suggest that targeting Notch signaling may be of therapeutic value in KS patients.

KSHV encodes two genes, K3 and K5, whose products are both type III integral membrane proteins containing a zinc-binding plant homeodomain (PHD) sequence at the N-termini, two hydrophobic transmembrane regions and a series of protein motifs important in cellular trafficking in the C-terminus (Coscoy and Ganem 2000; Ishido *et al.*, 2000; Means *et al.,* 2002; Sanchez *et al.*, 2002). The PHD domains of these proteins resemble those found in a number of E3 ubiquitin ligases and are capable of mediating self ubiquitinylation (Coscoy *et al.*, 2001). While the exact mechanism of MHC class I and CD54 down regulation is still only partially understood, this ability of K3 and K5 to act as an E3 ligase seems critical. The transmembrane regions of these two proteins probably define the target specificity. K3 is able to downregulate multiple HLA haplotypes, whereas K5 downregulates a much more restricted set. Downstream of the transmembrane regions, both contain a conserved series of residues identified as being important in protein:protein interactions and cellular trafficking (Means *et al*., 2002; Sanchez *et al.*, 2002). Without MHC class I on the cell surface, no peptides are presented to induce CTL activation and K3/K5 expressing cells are able to escape killing (Ishido *et al.*, 2000). While MHC I normally acts to transmit a negative signal to NK cells, the ICAM-1 and B7.2 molecules act as anchors to bring the NK cell into close conjugation with target cells. By removing these last two molecules from the surface of infected cells, K5 reduces the average time that the NK cell stays in contact with the K5-expressing target cell and thus, the NK cell releases the K5-expressing cell unharmed. Here, I demonstrate that cellular Notch signal transduction provides a novel expression profiling of KSHV K5 immune deregulatory gene, which leads to comprehensive evasion of host immune controls during viral latency.

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