Molecular Interaction between a Bcl-2 Homolog from Kaposi Sarcoma Virus and p53

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Many viruses have anti-apoptotic proteins to prevent apoptotic defense mechanism of virus-infected host cells. Since Bcl-2 family proteins are key players in the regulation of apoptosis, the homologs of Bcl-2 with structural and functional similarity are expressed by viruses such as Kaposi sarcoma-associated herpes virus (KSHV), adenovirus, and Epstein-Barr virus (EBV). For example, Kaposi sarcoma tumor-causing virus KSHV contains a Bcl-2 homolog called KSHV Bcl-2 and the protein protects virus-infected cells from apoptosis.[1] KSHV Bcl-2 has four conserved Bcl-2 homology (BH) domains encompassing BH1-BH4 and the presence of BH4 domain is characteristic of the human anti-apoptotic Bcl-2 family members (Fig. 1). Although KSHV Bcl-2 shares the overall fold as human Bcl-2, its specificity is different from that of human anti-apoptotic Bcl-2 family proteins.[2] While how the virus mimics host proteins is of great therapeutic importance, the mechanism of apoptosis inhibition by KSHV Bcl-2 is largely unknown.

Human p53 tumor suppressor has been known to induce cell cycle arrest and apoptosis of damaged cells. Aside from its transcription-dependent apoptosis, p53 mediates the mechanism of transcription-independent apoptosis. In response to various apoptotic stimuli, cytosolic p53 moves to the mitochondria and binds to Bcl-2 family proteins. In particular, the interactions of p53 with the anti-apoptotic Bcl-2 family proteins such as Bcl-2 and Bcl-XL were shown to relieve the inhibition of pro-apoptotic Bcl-2 family proteins by them, leading to activation of intrinsic mitochondrial apoptosis pathway.[3] Although it can be speculated that viral Bcl-2 homologs mimic the function of cellular anti-apoptotic Bcl-2 family proteins, any molecular interaction of viral Bcl-2 homologs with p53 has not been observed to date. In this study, we studied the molecular interaction between KSHV Bcl-2 and p53 TAD by NMR binding experiments.

Figs. 2A and 2B show the ^{1}H-^{15}N HSQC spectra for free state and KSHV Bcl-2-bound p53 TAD, respectively. The crosspeaks for free state p53 TAD at pH 7.8 were assigned using the previous assignment obtained at different pH.[4] Upon binding to KSHV Bcl-2, the crosspeak corresponding to Leu26 disappeared and the intensity of crosspeaks corresponding to Leu22, Trp23, Lys24, and Leu25 was greatly reduced. This spectral change seemed to arise from significant line broadening

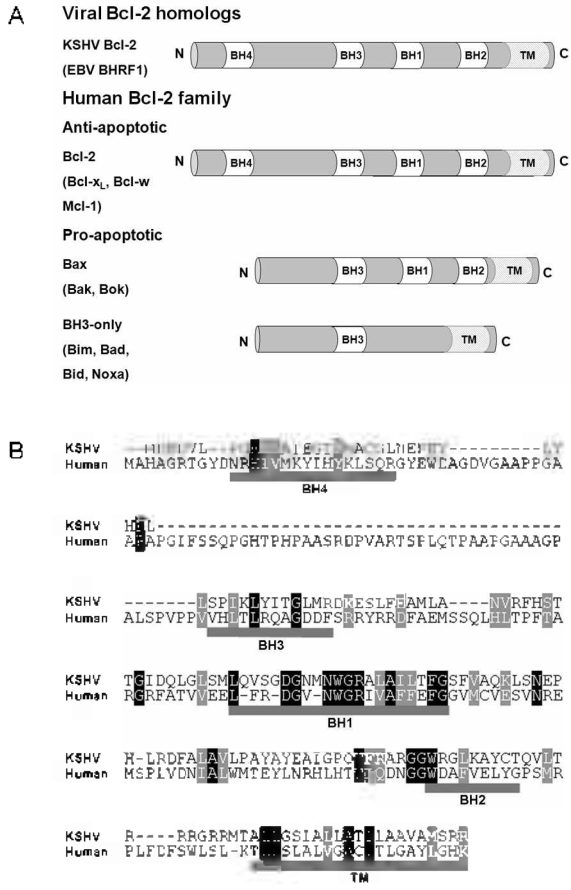

Figure 1. Comparison of domain structure and sequence between KSHV Bcl-2 and human Bcl-2 family proteins (A) Human Bcl-2 family proteins are classified into three subfamilies (Bcl-2, Bax, and BH3-only). Bcl-2 homology (BH) domains and transmembrane segment (TM) are indicated. (B) Sequence alignment between KSHV Bcl-2 and human Bcl-2. The BH domains and TM are shown below the sequence.

[a]These authors contributed equally to this work.

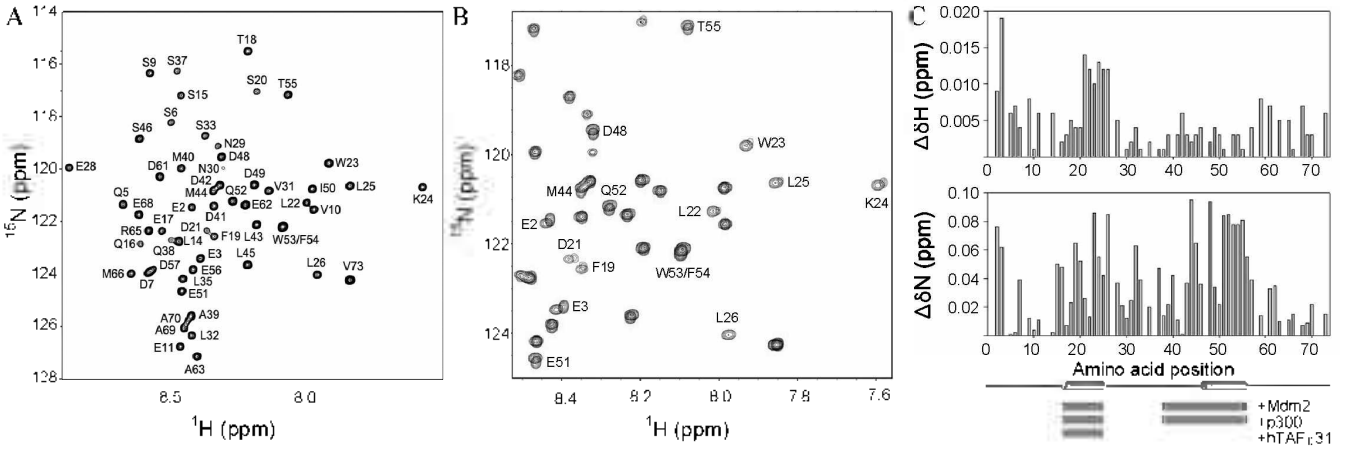

Figure 2. Chemical shift perturbations on p53 TAD by binding KSHV Bcl-2. (A) The 2D ^{15}N-^{1}H HSQC spectra for free state p53 TAD. (B) The overlaid HSQC spectra for free state (blue) and KSHV Bcl-2-bound p53 TAD (red). The crosspeaks of p53 TAD residues that showed significant reduction of intensity or chemical shift change upon binding KSHV Bcl-2 are labeled. To observe obvious chemical shift perturbation, binding titration was performed until the molar ratio of p53 TAD to KSHV Bcl-2 reaches 1:4.5. (C) Chemical shift perturbations on p53 TAD by binding KSHV Bcl-2 and binding site comparison. Chemical shift changes for ^{1}H (top) and ^{15}N frequency (bottom) are plotted against the residue number of p53 TAD. For comparison, the sites where significant NMR chemical shift perturbations occurred upon binding mdm2, hTAF$_{II}$31 or p300 are displayed in the bottom.[4,7,8] The cylinders in the bottom indicate the location of α-helices found in the structures of mdm2-bound and Tfb1-bound complexes.[9,10]

due to intermediate chemical exchange on the NMR time scale (Fig. 2B). This indicates that the residues 22-26 of p53 TAD are significantly involved in the interaction with KSHV Bcl-2. Figure 2 shows chemical shift perturbations on p53 TAD upon binding to KSHV Bcl-2. Except flexible N-terminal terminal residues 2-3, the largest chemical shift changes were observed at the two regions of p53 TAD encompassing the residues 18-26 and 44-57, indicating that they form the binding sites for KSHV Bcl-2. Interestingly, the KSHV Bcl-2-binding regions in p53 TAD are well overlapped with the sites where significant chemical shift perturbations occurred upon binding mdm2,[4] p300,[5] or hTAF$_{II}$31[6] (Fig. 2C). Due to difference in binding affinity, the magnitude in chemical shift perturbation for KSHV Bcl-2 is lower that that for mdm2. However, exchange broadening in residues 22-26 of p53 TAD is common for binding to mdm2, p300 and hTAF$_{II}$31. The agreement in the chemical shift perturbation data suggests that the binding of p53 TAD with KSHV-Bcl2 may occur in a manner similar to that with mdm2, p300, and hTAF$_{II}$31. While p53 TAD is structurally disordered, it is likely that the well-defined amphipathic α-helices in the p53 TAD (Fig. 2C), formed by residues 18-26 and 47-55, mediate hydrophobic interaction with KSHV Bcl-2 as observed in the structures of mdm2-bound and Tfb1-bound complexes.[7,8]

In this report, we show the first case where the direct interaction between viral Bcl-2 homolog and p53 is observed. Considering the structural similarity with KSHV Bcl-2, the other viral Bcl-2 homologs such as M11L from myxoma virus may also be involved in the direct interaction with p53 TAD. For cellular Bcl-2 family proteins, *in vivo* interaction of Bcl-2 and Bcl-XL with p53 was observed at the cellular level[9] and the interactions were shown to be mediated via the DNA-binding domain of p53.[3,10] Recently, Bcl-XL was observed to interact with the N-terminal domain of p53.[11] Therefore, it is possible that KSHV Bcl-2 prevents the transcription-independent apoptosis of p53 by interfering with the direct interactions between p53 and cellular anti-apoptotic Bcl-2 family proteins. Dysregulation of the host apoptosis mechanism by viral Bcl-2 homolog may enhance the survival of the virus-infected host cells. The information on the dysregulated pathway and the binding site should contribute to the development of antiviral therapies.

In conclusion, we demonstrated direct interaction between KSHV Bcl-2 and p53 TAD using NMR binding experiments. Our chemical shift perturbation data determined the KSHV Bcl-2-binding sites on p53 TAD at the atomic level, indicating that the binding sites coincide with those for mdm2, p300, or hTAF$_{II}$31. Our observation suggests that the other viral Bcl-2 homologs may be involved in the interaction with p53 as cellular Bcl-2 family proteins.

Experimental Section

KSHV Bcl-2 (residues 1-146), in which C-terminal transmembrane segment was truncated and N67D/V117A mutations were introduced, and p53 TAD (residues 1-73) were expressed and purified as previously described.[2,4] The 2D ^{15}N-^{1}H HSQC spectra of ^{15}N,^{13}C-labeled p53 TAD were obtained in the absence or presence of KSHV Bcl-2. All NMR data were acquired at 5 °C on Bruker Avance II 900 spectrometer equipped with cryogenic probe. NMR samples containing 50 μM ^{15}N,^{13}C-labeled p53 TAD alone or with KSHV Bcl-2 were prepared in 95% H_2O/5% $^{2}H_2O$, 20 mM TrisHCl, pH 7.8 and 5 mM DTT. All NMR data were processed and analyzed using an nmrPipe/nmrDraw and SPARKY software.[12,13]

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