Peptides
The synthetic peptides (and their biotinylated form) were obtained from Biosynthesis, Inc (Lewisville, TX) and stored lyophilized at -20C until reconstituted with sterile pure acidic water immediately before use (for both in vitro and in vivo experiments). The purity determined by HPLC-MS was 98% or higher for each peptide. The recombinant peptides were obtained and stored as described. The control peptide (CP) for R was previously published,[1] for S1 and S2 was NH_2-YGRKKRRQRRRG-OH, for S3 was NH_2-GLFGAIAGFIENGWEGMIDGGRKKRRQRRRG-OH, for S4, S5, and S6 was (D aminoacid isomers are shown between parentheses) NH_2-G(RRRQRRKKR)GG(DIM)G(EW)G(NEIF)G(AIA)G(FL)G-OH; and for S7 was NH_2-G(RRRQRRKKR)GG-OH. The mutated version for S1 was NH_2-YGRKKRRQRRRGGLVATVKEAGRAAHEIPREEL-OH, for S2 was NH_2-YGRKKRRQRRRGGRAAHEIPR-OH, for S3 was NH_2-GLFGAIAGFIENGWEGMIDGGRKKRRQRRRGGRAAHEIPRG-OH and for S6.2 was NH_2-G(RRRQRRKKR)GG(RGIEHAAR)GG(DIM)G(EW)G(NEIF)G(AIA)G(FL)G-OH. The aminoacid sequence for the TAT peptide was NH_2-YGRKKRRQRRRG-OH and for the Fusogenic peptide (Fu) was NH_2-GLFGAIAGFIENGWEGMIDG-OH.

Reporter Assays
Dual luciferase assays (Promega, Madison, WI) were performed in 293T cells transfected with 100 ng of either (GAL4)5-TK-LUC, $(BCL6)_4$-TK-LUC, (Kaiso)-TK-LUC, (HIC)-TK-LUC, (PLZF)-TK-LUC, and the corresponding expression vectors plus a renilla reporter as internal control, as published.[1] Cells were treated with 20 μM of RI-BPI or CP for 12 hours, and the luminescence was measured in a Polarstar Optima microplate reader (BMG Labtechnologies, Germany).

In vitro BPI binding
To determine the in vitro RI-BPI binding to BCL6, 10^7 Ly1 cells were lysed in modified RIPA buffer (50 mM Tris, 250 mM NaCl, 1% Nonidet P-40, 0.5% deoxycholate, 0.1% SDS) containing a protease inhibitor cocktail. Cell lysates were exposed to $RI\text{-}BPI^{biotin}$ or CP^{biotin} for 1 hour and incubated with anti-BCL6 (N3, Santa Cruz, Santa Cruz, CA) or anti-IgG (Santa Cruz) as control. The immunocomplex was pulled-down using protein-A agarose beads and eluted with loading buffer (SDS 1%, NaCO3H 100mM). The proteins were transferred to a PVDF membrane (Bio-Rad, Hercules, CA) and incubated with a solution of avidin-biotinylated-peroxidase (Vectastain ABC, Vector Laboratories, Burlingame, CA). The complex was visualized using luminol (Santa Cruz) as chemiluminescent substrate for peroxidase.

Chromatin Immunoprecipitation (ChIP) analysis
ChIP was made as previously described with modifications [2] briefly 10^8 Ly1 cells were treated for 4 hours with 20 μM of RI-BPI or CP, and then fixed with 1% formaldehyde. Cell lysates were sonicated using a Bioruptor (Diagenode Inc., New York, NY). BCL6 N-3 (Santa Cruz), SMRT (Upstate, Lake Placid, NY) and actin (Santa Cruz) antibodies were added to the pre-cleared sample and incubated overnight at 4C. Then, protein-A beads were added, and the sample was eluted from the beads followed by de-crosslinking. The DNA was purified using PCR purification columns (Qiagen, Valencia, CA). Quantification of the ChIP products was performed by quantitative PCR (DNA Engine Opticon 2, Bio-Rad) using SyBr Green (Applied

Biosystems, Foster City, CA). Primers were designed to verify binding of BCL6 and SMRT at possible BCL6/SMRT binding sites in the p53 locus based on the report of Phan et. al.[3]

Real-time PCR

Harvested tumors from xenotransplanted mice were kept in RNAlater (Qiagen) at -80C to stabilize RNA. We extracted RNA from 10^4 peptide-treated and control cells, and from about 20 mg of peptide-treated and control tumors using RNeasy kit (Qiagen) following the manufacturer instructions. We synthesized cDNA using Superscript First Strand cDNA synthesis kit (Invitrogen, Carlsbad, CA). We amplified GAPDH, CD20, and ATR using published primers sequences[2,4] and p53 with the following primers: 5′-AATCAACCCACAGCTGCAC-3′ and 5′-TCTTCTGTCCCTTCCCAGAA-3′. Thermal cycler conditions were: initial step of 10 min at 95C followed by 40 cycles of 15 sec at 95C (denature) and 1 min at 60C (anneal/extend). For the cell lines, the C T values of GADPH were subtracted from the correspondent genes of interest (ΔC T). The standard deviation of the difference was calculated from the standard deviation of the C T values (triplicates). Then, the ΔCT values of the peptide-treated cells were expressed relative to their respective control-treated cells using the ΔΔCT method. The folds of expression for each gene in cells treated with peptide/drug relative to control treated cells is determined by the expression: $2^{-\Delta\Delta CT}$. Results were represented as folds of expression with the standard error of the mean (SEM) for 3 series of triplicates. For tumor tissues, we construct a standard curve for CT values of each gene of interest (p53, ATR and GAPDH) by the cDNA amount (in ng) of an external calibrator (Ly1 cell line). Then, we calculated the amount of each gene of interest in the experimental samples. Results are expressed as normalized values (ng p53 / ng GAPDH and ng ATR / ng GAPDH) in arbitrary units.

Growth inhibition determination

DLBCL cell lines were grown at its respective concentration that were sufficient to keep the untreated cells in exponential growth over the 48 h peptide exposure time. We determined cell viability by using a fluorometric resazurin reduction method (CellTiter-Blue, Promega) following the manufacturer instructions. The fluorescence ($560_{Ex}/590_{Em}$) was determined using the Polarstar Optima microplate reader (BMG Labtechnologies). The number of viable cells in each treated well was calculated by using the linear least-squares regression of the standard curve. Optical density was determined for 6 replicates per treatment condition or standard. We normalized cell viability in peptide-treated cells to their respective controls. We verified cell viability by the Sulforhodamine B assay (Sigma, Milwaukee, WI) following manufacturer's instructions with minor modifications for suspended cells. Experiments were carried out in triplicates and data are represented as percentage of growth inhibition to respective control with 95% confidence interval. We used CompuSyn software (Biosoft, Great Shelford, Cambridge, UK) to plot the dose-effect curves and to determine the concentration a peptide that inhibits 50% the growth of cell lines compared to control peptide treated cells (GI_{50}).

Mice toxicity studies

Six to eight-week old male C57BL mice were purchased from the NCI and housed in a barrier environment. Peptides were stored, reconstituted and administered as before. RI-BPI and control were administered at 500 μg daily for the first 3 weeks and weekly for the following 49 weeks. Mice were weighed every other day during the 3-week period and weekly thereafter. All mice were euthanized by cervical dislocation under anesthesia accordingly to predetermined time

points (3-weeks and 1 year). At the moment of euthanasia, blood was collected and the organs were harvested and weighted. All organs and tissues underwent careful macroscopic and microscopic (hematoxilin-eosin staining) examination.

Mice germinal center models

Fifteen 8-week-old male C57BL mice were intraperitoneally injected with 0.5 ml 2% v/v sheep red blood cells (Cocalico Biologicals Inc). The day after the immunization, mice were randomized in 3 groups (n=5) and after 3 days were daily treated with vehicle, CP or RI-BPI 500 μg for 7 days. All mice were euthanized by cervical dislocation under anesthesia at day 10, and the whole spleen were removed, weighed, fixed, and embedded in paraffin. B cells were identified by a biotinylated anti-CD45R (Caltag, Invitrogen) and germinal center activated B cells by a biotinylated peanut agglutinin (Vector). Color was developed with diaminobenzoate chromogen peroxidase substrate (Vector). Pictures were taken using a color camera (AxioCam, Zeiss, Germany) attached to an AxioSkop II light microscope (Zeiss) and processed using ImageJ (NHI). PNA and CD45R clusters were counted and measured in each whole-spleen digitally reconstituted longitudinal sections.

Human β2-microglobulin

Serum levels of human β2-microglobulin were determined in the mice at day 10 by enzyme immunoassay (Quatikine IVD, R&D Systems, Minneapolis, MN) following the manufacturer instructions. The mean absorbance ($A_{450} - A_{620}$) values for each set of triplicates and standards were measured using the Polarstar Optima microplate reader (BMG Labtechnologies) and concentrations were calculated using a four-parameter logistic curve fit (SigmaPlot, Systat Software, San Jose, CA).

Apoptotic index

The DNA fragmentation coupled to the apoptotic response was detected in morphologically identifiable nuclei and apoptotic bodies present in formalin-fixed paraffin-embedded tumors by the TUNEL assay (ApopTag, Chemicon, Temecula, CA) following the manufacturer instructions with optimization. Tissue slides were pre-treated with 0.5% trypsin for 15 minutes (Zymed, San Francisco, CA), to improve the exposure of DNA.

Proliferation index

We identified PCNA by immunohistochemistry as previously described.[5] Briefly, deparaffinized slides were antigen retrieved in citrate buffer pH 6.0 (Zymed) then 1:1000 anti-PCNA (Santa Cruz) was applied, followed by incubation with a corresponding biotinylated-conjugated secondary antibody (Vector). We incubated slides with a preformed avidin and biotinylated horseradish peroxidase macromolecular complex, Vectastain ABC (Vector). Color was developed with diaminobenzoate chromogen peroxidase substrate (Vector). PCNA immunohistochemistry results were scored using ImageJ software (National Institutes of Health, Bethesda, MD).

Mitotic index

We identified mitotic cells by using standard hematoxylin and eosin stain of the tumors in pictures taken using a color camera (AxioCam, Zeiss) attached to an AxioSkop II light

microscope (Zeiss) and processed using ImageJ (NHI). The index represents the number of mitotic cells over total number of cells. A minimum of 1,000 cells were counted per tumor.

BPI distribution in tumors

We determined the amount of L-BPI^{biotin} and RI-BPI^{biotin} in the serum of mice by using the Quant-Tag Biotin kit (Vector) following the manufacturer instructions. Standards with known concentration of biotin were used to generate a curve to calculate the concentration of biotin in the serum samples. HPLC-MS analysis indicated that 1mol of biotin was bound to 1mol of peptide.

Serum kinetic of BPI

L-BPI^{biotin} and RI-BPI^{biotin} peptides were detected by histochemistry in tumor tissues using Texas red-avidin (Vector). Briefly, tumor sections were deparaffinized and hydrated trough xylene and graded ethanol series, followed by rinses and incubation with TR-avidin 1 h at RT in buffered saline solution pH 8.2. Slides were mounted with permanent mounting medium (Vectashield Hard set, Vector) to prevent photobleaching and pictures were immediately taken using a color camera (AxioCam, Zeiss) attached to an AxioSkop II fluorescent microscope (Zeiss).

Immunogenicity studies

Serum from C57/black mice treated with RI-BPI and controls were stored at -80C until used. To detect anti-RI-BPI antibodies present in the serum we performed ELISA arrays in streptavidin-coated plates (Reacti-Bind, Pierce, Rockford, IL). Briefly, these or L-BPI^{biotin} plates were incubated with three different concentrations of RI-BPI^{biotin} diluted in 1% BSA followed by incubation with 10 different triplicates dilutions (1:10 to 1:10,000) of serum and controls. Then, 50 µL of alkaline phosphatase conjugated goat-IgG anti-mouse-IgG (Zymed) in triplicates (in 1% BSA) were added to each well. The plates were developed using p-nitrophenyl phosphate as substrate solution (Sigma) and read at 405 nm using the Polarstar Optima microplate reader (BMG Labtechnologies).

Statistics

The comparisons between treated and control mice were done using two-tailed T-test (Statistix, Analytical Software, Tallahassee, FL). Survival time was considered as the time elapsed (in days) from the start of the treatment (T_0) until death or until the tumor volume increase 10 times from T_0 (whatever event occurs first). Survival curves were calculated using the Kaplan-Meier method and groups were compared using Gehan-Wilcoxon test for multiple samples and Cox's F test for two-groups comparisons (Statistix). Serum concentrations of human β2-microglobulin in mice were correlated with tumor weight using the Pearson's coefficient (Statistix).

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