Supplementary Materials and Methods

Cell culture.

Primary human keratinocytes were isolated from human foreskin tissue and were maintained in keratinocyte serum-free medium. Primary human umbilical vascular endothelial cells (HUVEC) were cultured in Medium 199 (Invitrogen, Carlsbad, CA) supplemented with 20% fetal bovine serum (FBS), endothelial cells growth supplement (ECGS, 20 ng/ml), and 2 mM L-glutamine. The Huh-7 (human hepatocellular carcinoma) cell line was obtained from the Health Science Research Resources Bank (Osaka, Japan; JCRB0403). BHK (baby hamster kidney), CT26 (mouse colon cancer), BNL (mouse transformed liver cells), Hep3B (human hepatocellular carcinoma), HeLa (human cervical cancer), LNCaP (human prostate cancer), HEK293, 293T cell lines, Hepa 1-6 (mouse hepatoma), LL/2 (mouse lung carcinoma), EL4 (mouse lymphoma), N1-S1 (rat hepatoma), A549 (human lung carcinoma), U2OS (human osteosarcoma), HepG2 (human hepatoma), and T98G (human brain glioblastoma) were obtained from the American Type Culture Collection (ATCC, Manassas, VA). BNEF cells were isolated from the 14-day-old embryos of Brown Norway rats. GP7TB cell line was derived from chemically transformed hepatic epithelial cells of Fischer 344 rat.[1] RT-2 cell line was derived from an avian sarcoma virus-induced brain tumor in Fischer 344 rat.[2] MBT-2 is a mouse transitional cell carcinoma cell line induced by carcinogen *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide and is provided by Dr. Ming-Derg Lai

(National Cheng Kung University, Tainan, Taiwan). YD-SML and YD-SML-tk lung cancer cell lines, provided by Dr. Win-Ping Deng (Taipei Medical University, Taipei, Taiwan), were derived from the FVB mouse and maintained in MEM supplemented with 10% FBS. LNCaP cells were cultured in RPMI 1640 supplemented with 10% FBS. N1-S1 cells were maintained in IMDM supplemented with 10% FBS. A549 cells were maintained in F-12K medium supplemented with 10% FBS. All the other cell lines mentioned above were maintained in DMEM supplemented with 10% FBS.

Flow cytometric analysis of laminin receptor.

To determine the surface expression of laminin receptor, a monoclonal antibody, MLuC5 (Enzo, Farmingdale, NY), was used as previously described.[3] The FITC-conjugated anti-mouse IgM antibody (1:50 dilution, eBioscience, San Diego, CA) was used as a secondary antibody.

Northern blot analysis of viral RNA species.

Total cellular RNA from the indicated cell lines with or without SBV infection was extracted using TRIZOL reagent (Invitrogen). Five microgram of the RNA were denatured with glyoxal/DMSO and electrophoresed on a 1% agarose gel. A probe containing the SBV *E1* structural gene and a GAPDH probe were generated and used for hybridization using the DIG

System (Roche, Indianapolis, IN) following the manufacturer's instructions.

Lentivirus-mediated gene knockdown or overexpression.

The lentiviral clones expressing shRNA targeting human MDA5, STAT1, and mouse IFNAR1 were purchased from the National RNAi Core Facility, Academia Sinica, Taiwan. The lentiviral particles expressing shRNA or human MDA5 cDNA were produced by transfecting the vectors along with the packaging plasmid *pCMV-ΔR8.91* and the envelope plasmid *pMD.G* into 293T cells using a calcium phosphate precipitation method. The knockdown or overexpression experiments were performed as following: cells were incubated with lentiviruses at an MOI of 3 and polybrene at a concentration of 8 μg/ml. After an overnight incubation, the medium was replaced with fresh medium containing selection drugs. Cells were then examined for the expression of the indicated genes or for further experiments.

Real-time quantitative RT-PCR.

Total cellular RNA from cells was extracted using TRIZOL reagent (Invitrogen) and 1 μg of RNA was subjected to reverse transcription. Twenty five ng of the cDNA were then incubated with specific primers using 2x FAST CYBR reagent (Applied Biosystems, Carlsbad, CA) and quantitative PCR was performed on a StepOnePlus Real-Time PCR

System (Applied Biosystems). The reaction mixture was first denatured at 95°C for 20 sec and then 40 cycles of PCR were performed using the following settings: 95°C, 3 s; 60°C, 30 s. The expression levels of each gene were normalized with those of β-actin (for mouse genes) or GAPDH (for human genes).

Immunohistochemical staining and TUNEL assay in the tumor regions.

Tumor tissues were removed, embedded in Tissue-Tek OCT compound, snap-frozen, and then sectioned into 5 μm-thick cryosections. The sections were stained for CD8 or CD4 with rat monoclonal anti-mouse CD8 or anti-mouse CD4 antibodies, respectively (1:50 dilution, BD Pharmingen, San Diego, CA), incubated with rabbit anti-rat IgG (1:200 dilution), and then developed using EnVision+ system-HRP (Dako, Carpinteria, CA) according to the manufacturer's instructions. Apoptotic cells were detected by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using the *In Situ* Cell Death Detection Kit (Roche), following the manufacturer's instructions.

In vitro cytotoxic T lymphocyte assays.

Spleens were isolated from the SBV-infected animals and homogenized in complete RPMI medium to obtain single cell suspensions. ML-1_{4a} or ARKD cells in suspension were treated with mitomycin C (50 μg/ml) for 20 min at 37°C. After mitomycin C treatment, the

cells were washed three times with medium and then used as stimulator cells. The stimulator cells (1 x 10^5) were incubated with splenocytes (5 x 10^6) in the presence of recombinant human IL-2 (20 ng/ml) for 5 days. After incubation, the splenocytes were recovered and used as effectors; they were co-cultured with target cells (2 x 10^3 ML-1_{4a}, ARKD, or BNL cells) at an effector/target ratio of 10:1, 33:1, or 100:1. Specific cytotoxic activity was measured 4 h after co-culture by the release of lactate dehydrogenase (LDH) using the CytoTox 96 LDH kit (Promega, Madison, WI) according to the manufacturer's instructions

Depletion of CD8$^+$ T cells.

CD8$^+$ T cells were depleted by intraperitoneal injection of 0.5 mg of anti-CD8 monoclonal antibody (53-6.72) on days 5, 7, 9, 11, 14, 21, 28, and 35 after ARKD tumor implantation. An irrelevant rat monoclonal IgG2a isotype antibody was used as a control which was injected at the same dose and schedule as the anti-CD8 antibody. One day after the last antibody injection, the efficiency of CD8$^+$ T cells depletion was determined by flow cytometric analysis.

References

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