Supporting Information

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SI Materials and Methods

Cell Lines, Antibodies, and Cytokines. H1299, H441, A549, and U2OS cell lines were obtained from American Type Culture Collection. A375 and NHF cell lines were kindly provided by Isaiah J. Fidler, The University of Texas, MD Anderson Cancer Center, Houston, and Jieyu Zhu, Washington State University, Spokane, WA, respectively.

Recombinant IL-2 was acquired from AIDS Research and Reference Reagent Program. TNF-α was purchased from R&D Systems, and LPS was from Sigma. Antibodies for perforin and granzyme B were from Santa Cruz Biotechnology, and anti-β-actin was from Sigma. Anti-phospho-Stat proteins, anti-TLRs, and anti-Bcl-2 family proteins were obtained from Cell Signaling. The NKG2D–blocking antibody, the isotype IgG control antibody, and various allophycocyanin (APC)- or FITC-labeled antibodies were purchased from BioLegend. The HLA-A2–blocking antibody was purchased from GeneTex. GM-CSF-Fc4 and IL-4-Fc4 proteins were generated from 293 cells transduced with the lentivirus fusion gene constructs using PCR-based cloning method with the cDNAs of GM-CSF, IL-4, and IgG4 obtained from Open Biosystems.

Lentiviral Vector, Viral Production, and Transduction. The *tax* gene from HTLV-2 was fused with the fragment encoding enhanced green fluorescence protein, and the *tax2-gfp* fusion fragment was cloned into the lentiviral vector in which the human elongation factor promoter drives the expression of transgene. The lentiviral vectors that express HLA-A2 (cDNAs from A375 cells), B2M (cDNA from normal lymphocytes), and MICA (cDNA from A375 cells) were constructed using the PCR-based cloning method. To generate the fusion gene construct pTERT (amino acids 301 to 700), IκBα (cDNA from normal lymphocytes) and hTERT (amino acids 301 to 700) (cDNAs from U2OS cells) were amplified by high-fidelity PCR, enzyme-digested, and inserted into the lentiviral vector.

To generate recombinant lentiviruses, the lentiviral construct was cotransfected with the packaging plasmid mix containing the expression plasmids for VSV-G, Gag-Pol, and Rev (Invitrogen) into 293 cells using SuperFect transfection reagent (Qiagen). The viral supernatants were collected and were subjected to ultracentrifugation at 66,549 × *g* at 4 °C for 2 h. The virus pellets were resuspended and stored at −80 °C.

To generate target cell lines expressing luciferase, A375, H1299, A549, H441, U2OS, and NHF cells were transduced with the luciferase lentivirus at the multiplicity of infection (MOI) of 10. The expression of luciferase in these cell lines was verified by the luciferase activity assay using the kit from Promega according to the manufacturer's recommended protocol. The pTERT-expressing DCs were generated by the pTERT lentiviral transduction in ihv-DCs at the MOI of 20.

Generation of ihv-DC Cell Lines. Leukopaks were obtained from New York Blood Center. Human PBMCs were isolated from leukopaks and stimulated with PHA (5 μg/mL) for 24 h, followed by adding recombinant IL-2 (100 units/mL). The activated PBMCs were cultured for 4 to 5 d and were then transduced with the Tax2-GFP lentivirus in the presence of polybrene (10 μg/mL). The transduced cells were cultured continuously in the complete RPMI 1640 medium containing 10% FBS (Sigma) and 100 units/mL recombinant IL-2. Alternatively, the transduced cells were cultured in RPMI 1640 medium supplemented with 5% heat-inactivated human AB serum (Sigma). Two- to 3-wk after transduction, the transduced cells were negatively selected with anti-CD3 magnetic beads (Life Technologies) to deplete T cells. CD3-negative cells were maintained in culture continuously and analyzed for their immunophenotypes about 3 mo after transduction. Two dendritic cell lines (ihv-DC1 and ihv-DC2) were established from two blood donors. The ihv-DC1 cells lost CD40 during the passages in culture, and CD40 was restored by the CD40 lentiviral transduction. The ihv-DCs were grown in the RPMI 1640 medium supplemented with 5% heat-inactivated human AB serum or 10% FBS in the presence of IL-2 (50 to 100 units/mL).

To generate MoDCs, adherent monocytes from 4 million PBMCs were stimulated with GM-CSF-Fc4/IL-4-Fc4 using the conditioned medium from the GM-CSF-Fc4/IL-4-Fc4–producing 293 cells at 1:10 dilution for 7 d. The differentiated DCs were subsequently transduced with the Tax2-GFP lentivirus. The Tax-expressing MoDCs (MoDC-Tax) were cultured in the RPMI 1640 medium with 5% heat-inactivated human AB serum in the presence of GM-CSF-Fc4/IL-4-Fc4 for 5 to 7 d, and then continued to be maintained in culture in the presence of recombinant IL-2 at 100 units/mL The activated MoDCs were generated with stimulating the GM-CSF-Fc4/IL-4-Fc4–differentiated MoDCs with TNF-α (50 ng/mL) and LPS (1 μg/mL) for 2 d. The mature and activated MoDCs showed typical dendrites and were harvested for RNA and protein extractions.

Immunophenotyping Analysis by Flow Cytometry. The immunophenotypes of ihv-DCs cells, MoDC-Tax, and DC-activated lymphocytes were analyzed with FACS. Cells were stained with APC-conjugated antibodies (BioLegend), as indicated in the figures according to the manufacturer's instructions. The stained cells were subjected to FACS analysis. For TLR3 intracellular staining, the ihv-DCs were stained with APC-conjugated anti-TLR3 antibody or IgG isotype control antibody after permeabilization using the intracellular staining kit from eBioscience according to the manufacturer's instructions.

Induction of Tumor Antigen-Specific CTLs by ihv-DCs. The ihv-DCs were mixed with naïve PBMCs isolated from leukopaks at the ratio of 1:100. The mixed cells were kept in culture without adding exogenous cytokines for 2 to 3 d, followed by adding recombinant IL-2 (100 to 200 units/mL). The proliferation of the ihv-DC–reactive lymphocytes was monitored with FACS, and the presence of ihv-DCs in the mixed culture was monitored using fluorescence microscopy and qRT-PCR for detecting Tax2. Two to 3 wk after cell mixing, the ihv-DC–activated lymphocytes were analyzed with FACS and examined for their cytotoxic activity on target cells.

Cytotoxicity Assay. Various cell lines that were modified to express luciferase were used as targets, while the ihv-DC–activated lymphocytes generated from 14 to 21 d after mixed lymphocyte reaction were served as effectors. Cancer cells were first placed in 24-well plates for 2 h for complete attachment. The ihv-DC–activated lymphocytes were then placed on cancer cells at the indicated E:T ratios. At the 4- or 16-h time point after coincubation of effector and target cells, viable cells were gently washed with PBS buffer to remove cellular debris and subjected to the luciferase activity assay using the kit from Promega. The cytotoxic activity of the ihv-DC–activated lymphocytes was determined by comparing the luciferase activities in the target cells that were treated without or with the cytotoxic lymphocytes at the indicated E:T ratios.

Electrophoretic Mobility-Shift Assay (EMSA). Nuclear extracts were prepared from various cell lines using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce). The oligonucleotide was 5′-end–labeled with biotin (Integrated DNA Technologies) and annealed to its complementary strand. The binding activities were examined by EMSA using a light-shift chemiluminescent EMSA kit (Pierce) following the protocol reported previously (22). The oligonucleotide probes are for STAT3 (5′-GATCC-TTCTGGGAATTCCTAGATC-3′), NF-κB (5′-GAT-CCGGCAG-GGGAATCTCCCTCTC-3′), and AP-1 (5′-CGCTTGATGACT-CAG-CCGGAA-3′).

Immunofluorescence Imaging. Cells were washed with PBS and fixed for 20 min with 4% paraformaldehyde and 10 min with 0.1% Triton X-100. After washing, cells were incubated with 5% goat serum albumin as the blocking solution. The primary antibody anti-FLAG (1:100; Sigma) was applied, followed by the secondary antibody, anti-rabbit Alexa Fluor 488 (Cell Signaling), at the dilution of 1:200. After washed for three times with cold buffer (mixture of Tris-buffered saline and Tween 20), and the cell nuclei were stained with DAPI (1:2,000; Cell Signaling). The stained cells were examined using fluorescence microscopy (Nikon E800).

qRT-PCR. Total RNA was isolated using the RNeasy kit (Qiagen), and its concentration was determined using the NanoDrop1000 spectrophotometer (Thermo Scientific). The quality and integrity of total RNA was assessed on 1% formaldehyde–agarose gels. cDNA was synthesized using the Omniscript Reverse Transcriptase Kit (Qiagen) following the manufacturer's recommended protocol. Template samples in triplicate were subjected to the qRT-PCR (Stratagene Mx3005P system) using Power SYBR Green (Applied Biosystems). The primer sequences are listed in Table S1.

Animals and Xenograft Models. The animal study was performed using the approved Institutional Animal Care and Use Committee protocol of the University of Maryland School of Medicine. Male NSG mice (8 wk, 18 to 22 g body weight) were obtained from the animal center at the University of Maryland School of Medicine and housed in a specific pathogen-free room. A549/A2.1 cells (2 million) were collected, resuspended in Hanks buffer, and then tail vein-injected into NSG mice. After cancer cell implantation, the mice were randomized into two groups (CTL Tx and control). The treatment began 5 d after cancer cell transplantation. The treatment group mice were injected via the tail vein of mice with the DC-activated CTLs (q.5.d × 12, 20 million in 200 μL plus 1,000 u recombinant IL-2), and the control group mice were injected via the tail vein of mice with Hanks buffer (q.5.d × 11, iv, 200 μL plus 1,000 u recombinant IL-2). The animals' weight was measured every 2 d.

Immunohistochemistry and H&E Staining. Tissue sections were incubated at 60 °C for 10 min and then deparaffinized in xylene and subsequently in ETOH and deionized water. After deparaffinization, tissue sections were immersed into a preheated antigen unmasking solution (Vector), placed into a pressure cooker for 5 to 10 min, and then cooled to room temperature under cold water. The anti-human CD3 antibody or anti-NKp46 (1:100) was used to stain the pretreated tissue sections. Next, the endogenous peroxidase activity was blocked by incubation of tissue sections with 3% hydrogen peroxide in PBS for 10 min. Biotinylated goat anti-mouse IgG Antibody (H^+L) (1:200) (Vector) was applied as the secondary antibody. A VECTASTAIN Elite ABC HRP Kit (Vector) was used as chromogen, and hematoxylin counterstaining was also performed. H&E staining (paraffin-embedded) was conducted by the Pathology Electron Microscopy and Histology Laboratory, University of Maryland School of Medicine.

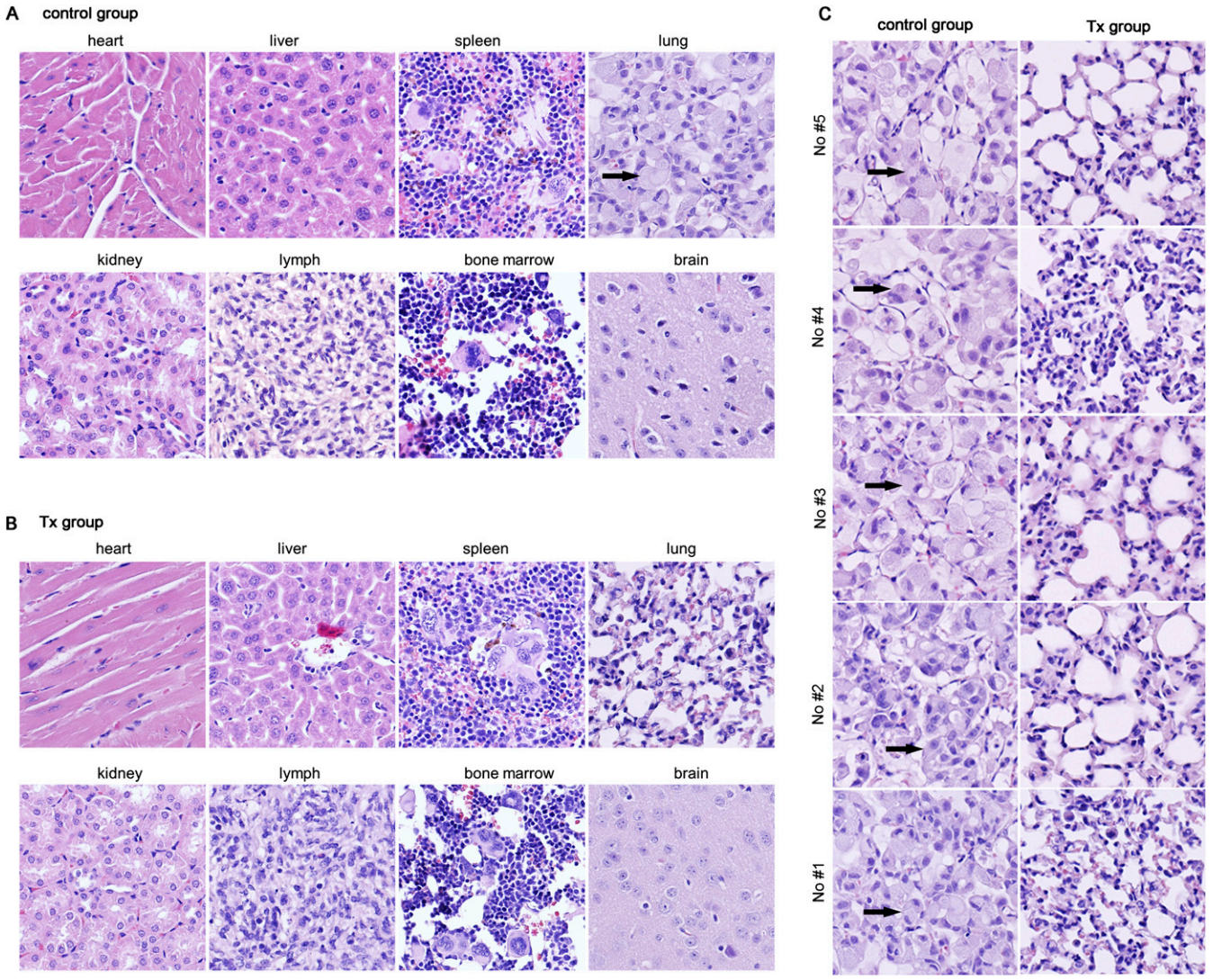

Fig. S1. Detection of A549-Luc/A2.1 cancer cells in NSG mice after the therapy by ihv-DC1.2-MAGEA3–activated cytotoxic lymphocytes. H&E staining of various tissues of mice in the control group (*A*) and the Tx group (*B*). (*C*) H&E staining of the lung tissues of all five mice in the control group (*Left*) and the Tx group (*Right*).

Table S1. Primers for qRT-PCR analysis

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
GAPDH	GCACCGTCAAGGCTGAGAAC	TGGTGAAGACGCCAGTGGA
IL-1A	CAGTGCTGCTGAAGGAGATG	AACAAGTTTGGATGGGCAAC
IL-1B	TGGCAATGAGGATGACTTGT	TGGTGGTCGGAGATTCGTA
IL2	CTCACCAGGATGCTCACATTTA	CCTCCAGAGGTTTGAGTTCTTC
IL6	GGAGACTTGCCTGGTGAAA	CTGGCTTGTTCCTCACTACTC
IL8	CTGGCCGTGGCTCTCTTG	CCTTGGCAAAACTGCACCTT
IL10	TTTCCCTGACCTCCCTCTAA	CGAGACACTGGAAGGTGAATTA
IL-12A	CCTTGTGGCTACCCTGGTCCTC	CTCAGCAGGTTTTGGGAGTGGT
IL12B	ACCAGAGCAGTGAGGTCTTA	CTCCTTTGTGACAGGTGTACTG
IL15	GAGTCCGGAGATGCAAGTATTC	CCTCCAGTTCCTCACATTCTTT
IFNA1	ACCCACAGCCTGGATAACAG	ACTGGTTGCCATCAAACTCC
IFNA2	TCAATCTCTTCAGCACAAAGGA	ATCACACAGGCTTCCAGGTC
IFNB1	TCTCCTGTTGTGCTTCTCCA	GTCAAAGTTCATCCTGTCCTTG
IFN-γ	CCCATGGGTTGTGTGTTTATTT	AAACCGGCAGTAACTGGATAG
TNF-α	GATCCCTGACATCTGGAATCTG	GAAACATCTGGAGAGAGGAAGG
MAGEA1	GCCTTTCCCACTACCATCAA	GAACAAGGACTCCAGGATACAA
MAGEA3	GTTCCAAGCAGCACTCAGTA	CCAGCATTTCTGCCTTTGTG
MAGEA4	TTTCTGCTCCGCAAGTATCG	TGCCGAAGATCACAGGAAAG
MAGEA6	CCAAGACAGGCTTCCTGATAAT	CCTCAAACACCTCTAACACACT
MAGEA10	TCGTTGCTTCCCTTCCATTAG	CTTCTGGGTAAAGACTCACTGTC
MAGEA12	GATCCTGCATGCTACGAGTT	TGAGGTCCTCCACTGATCTT
MAGEB2	CACCTTCATCGACAAGGTAGAC	AGAAGATCACACCCAGGAGA
MAGEB3	GCACTATCCACCACTACATCTG	GATAGACCCTGAGAGAAGCTTTG
CTAG1B	GTCCGGCAACATACTGACTATC	GTGATCCACATCAACAGGGAA
GPC3	GTGTCTCTCTACATCTTGGGTTC	CTGGGTTCATTAGCTGGGTATAG
CEACAM5	CAACAGGACCCTCACTCTATTC	CGCTGTGGTCAACACTTAATTC
MSLN	CCATTGGACCTGCTGCTATT	CGTGATGCGGGAGAAGAAAT
CT-83	GGCCCAAAGTTTCCCAAATC	GCTGCTCGCTAGGAGTAAATAG