**Supplemental materials and methods**

**Construction of Delta-24-RGDOX and propagation of the viruses**

First, DNA coding for the RGD-4C motif was introduced via site-directed mutagenesis into the region coding for the fiber HI loop of fiber protein (1) using a shuttle vector, pAB26 (Microbix Biosystems Inc.), resulting in plasmid pAB26-RGD. The mouse OX40L (mOX40L) (Origene) was subcloned into the KpnI/XbaI site in pcDNA3.1(+) (Life Technologies), and then the expression cassette for mOX40L (including the cytomegalovirus promoter and bovine growth hormone polyadenylation sequences) was subcloned into the ClaI/BamHI site (in the place of the *E3* region) of pAB26-RGD, producing pAB26-RGD-mOX40L. The final adenoviral genome was generated by homologous DNA recombination of pAB26-RGD-mOX40L and SwaI-linearized pVK500C.Delta-24 with a deletion of 24 bp of DNA coding for the RB-binding region (CR2) in the *E1A* gene in *Escherichia coli*, BJ5183 (2, 3). To rescue the Delta-24-RGDOX (Delta24-RGD-mOX40L) virus, the resulting viral backbone vector was digested with *Pac*I and then transfected into 293 cells with X-tremeGENE HP DNA transfection reagent (Roche Diagnostics Corporation). Thus, the resulting virus, Delta-24-RGDOX or Delta-24-RGD-GREAT, contained the following modifications: replacement of the *E3* region of the human adenovirus type 5 (hAd5) genome with mOX40L expression cassette; deletion of 24 bp in the *E1A* gene; and insertion of an RGD-4C motif-coding sequence in fiber gene (1, 4). The modification of the viral genome was confirmed through amplification of the modified region by polymerase chain reaction and then sequencing the products. The viruses were propagated in A549 cells (replication-competent viruses) or 293 cells (replication-deficient AdGFP) (5), purified by the Adenopure kit (Puresyn, Inc.), and stored at −80°C. The viral titer was assayed with the Adeno-X-Rapid Titer Kit (Clontech) and determined as PFU/ml.

**Viral replication assay**

Cells were seeded at 5 × 104 cells per well in 12-well plates and infected with the virus at 10 PFU per cell. Forty-eight hours after infection, the titers of the infectious viral progenies in the whole culture were determined using the Adeno-X-Rapid Titer Kit (Clontech) according to the manufacturer's instructions. Final viral titers were determined as PFU per milliliter.

**Immunoblotting**

The cells were collected and resuspended in PBS plus protease inhibitor cocktail (Sigma-Aldrich) and then subjected to lysis by adding an equal volume of 2× sodium dodecyl sulfate loading buffer. Then the lysates were heated at 95°C for 10 min. Equal amounts of proteins from the lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to a nitrocellulose membrane, and probed with antibodies as follows: rabbit anti-LC3B (Cell Signaling Technology), rabbit anti-E1A (Santa Cruz Biotechnology) and mouse monoclonal anti-α-tubulin (Sigma-Aldrich). Finally, the protein bands were visualized using an ECL Western blot detection system (Amersham Pharmacia Biotech, Piscataway, NJ) and the intensity of the protein bands were quantified with UN-SCAN-IT software (Silk Scientific, Inc.).

**Histopathological analysis of the brain tumors**

The brains were isolated from the euthanized mice, fixed with formalin, and embedded in paraffin wax. The whole-mount coronal sections of the brain were stained with hematoxylin and eosin following conventional procedures (6)*.*

**References**

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