Molecular Medicine

Supplemental Data

Guizhi Fuling Wan as a Novel Agent for Intravesical Treatment for Bladder Cancer in a Mouse Model

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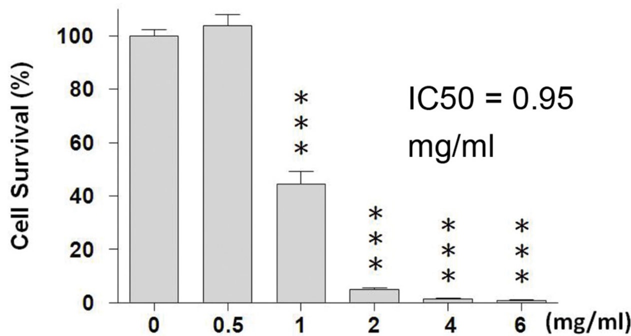
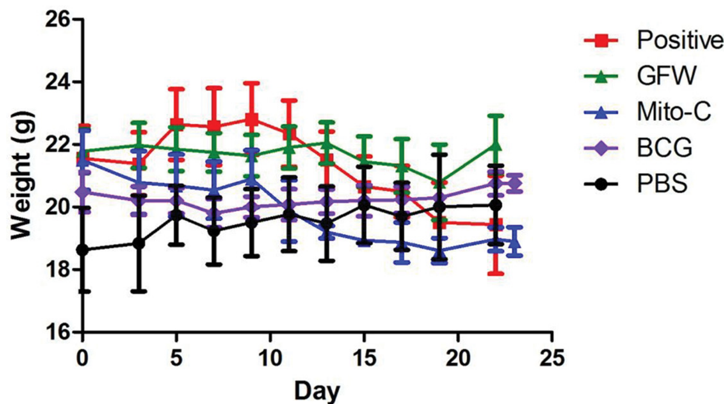
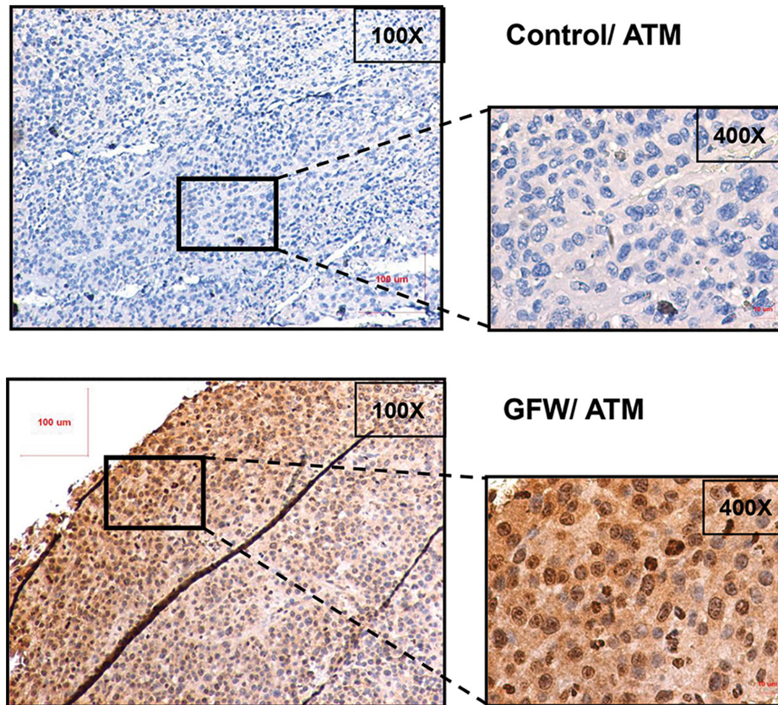


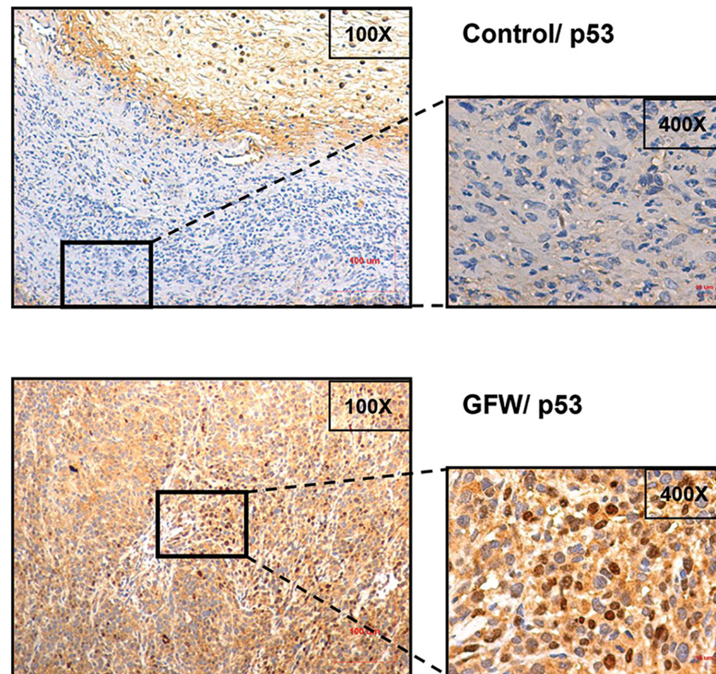
Supplementary Figure S1. Cytotoxicity of GFW against mouse urothelial cell line, MB49: Cells were initially seeded in 96-well plates at 1×10^4 cells per well and cultured for 24 h. The cells were subsequently starved in medium without FBS supplementation for 24 h, and then treated with agents at various concentrations for 24 h. Cell viability was detected using the Cell Counting Kit-8 (CCK-8). Data are presented as mean ± SEM (n=3). Significant differences from the control are indicated by * * * ($p<0.001$), as determined by one-way ANOVA and Dunnett's comparison test.



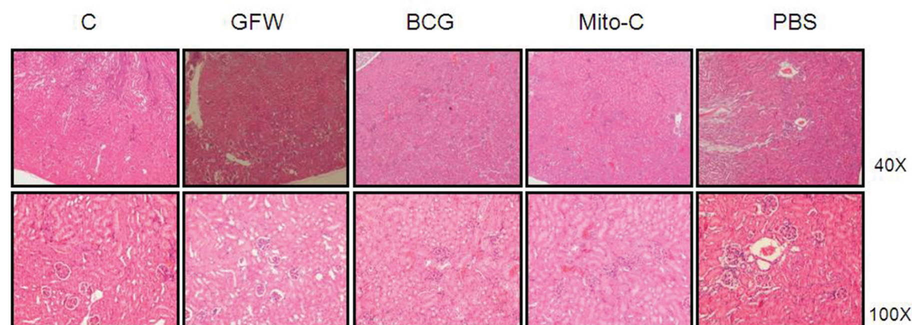
Supplementary Figure S2. Body weight of mice in five experimental groups. Except for the positive control which showed a slight decrease in body weight from d 10 after the tumor implant, all other groups showed a steady body weight throughout the experiment. Positive, MB49 only.



Supplementary Figure S3. Representative images of immunohistochemistry (IHC) examination of ATM in orthotopic mouse tumor. Upper panel, untreated control (MB49 only); lower panel GFW-treated group. Activation of ATM was observed in GFW-treated group but not untreated control.



Supplementary Figure S4. Representative images of immunohistochemistry (IHC) examination of p53 in orthotopic mouse tumor. Upper panel, untreated control (MB49 only); lower panel GFW-treated group. Activation of p53 was observed in GFW-treated group but not untreated control.



Supplementary Figure S5. Kidney sections stained with Hematoxilin & Eosin staining in the murine orthotopic bladder tumor model: Following the treatment cycle, the mice were sacrificed. All kidney tissue was stained with Hematoxilin & Eosin for histopathology. The above images present no evidence of tumor cells or lesions in any of the kidney sections. These results indicate that no metastasis occurred and that intravesicle treatment with these various agents was harmless to the kidney.

PROCEDURES USED IN SUPPLEMENTAL EXPERIMENTS

Cell Viability Assay

The effect of GFW on the growth of MB49 cells was determined using the Cell Counting Kit-8 (CCK-8) (SIGMA, Switzerland). Cells were initially seeded in 96-well plates at 1×10^4 cells per well and cultured for 24 h. They were subsequently starved in medium without FBS supplementation for 24 h, and then exposed to GFW of various concentrations (0.5, 1, 2, 4, or 6 mg/ml) for 24 h. Following GFW treatment, 10 μL of CCK-8 solution was added to each well and the plates were incubated at 37 °C for 1 h. The light absorbance of the culture medium in each well was measured at 450/655 nm using microplate reader Model 680 (Bio-Rad). We calculated the cell viable rate compared to the control (untreated, 0 mg/ml) using the following equation. The percentage of viability versus the concentration of a given agent was used to calculate the concentration that resulted in 50% cell viability (IC50): Viability (%) = 100 × Absorbance of treated group / Absorbance of untreated group.

Immunohistochemistry

Paraffin-embedded mouse bladder tumor specimens were assessed for the ATM and p53 expression. Briefly, 5-μm-thick sections of formalin-fixed paraffin-embedded specimens were mounted on poly-L-lysine–coated slides, deparaffinized for antigen retrieval and then rehydrated through graded alcohols to water. Retrieval buffers consisted of Tris-EDTA buffer pH 9 for p53, 10 mM citrate buffer pH 6 for ATM. Endogenous peroxidase activity was inhibited with 3% hydrogen peroxidase for 10 min. The primary antibody was anti-p53 monoclonal antibody clone PAb240 (dilution 1:200, Abcam) or anti-ATM polyclonal antibody H-300 (dilution 1:750, Santa Cruz) incubated at 4°C overnight, followed by the Polink DS-MR-Hu kit (GBI Labs, Burlington, Ontario). The sections were then counterstained with hematoxylin before mounting.