Electronic Supplementary Material (ESI) for Food & Function.

SUPPLEMENTARY METHODS

Cell viability assay

H22 cells (5×10^4 cells/well) were seeded in 96-well plates and then cells were treated with different concentrations of TB-TF (0, 5, 10, 20, 40, 80, 160 μg/mL) for 48 h, 10 μL of Cell Counting Kit-8 (CCK-8) solution was added to each well and then cells were cultured at 37 ºC for another 4 h. The absorbance was measured at 450 nm using SpectraMax M5 plate reader.

Flow cytometry analysis

H22 cells (5×10^5 cells/well) were seeded in 6-well plates and then cells were treated with or without TB-TF (5, 10, 20, 40, 80 μg/mL) for 48 h. Cells were collected and fixed with precooled 95 % ethanol at 4 ºC for 12 h. Then, cells were stained with propidium iodide (PI) without light for 30 min. The H22 cell cycle was detected by flow cytometry. Annexin V-FITC/PI was added to stain the cells for 15 min in dark. Cell apoptosis was detected by flow cytometry, and the data were analyzed using CytExpert software. Anti-mouse CD3-FITC antibody, anti-mouse CD4-PE antibody, and anti-mouse CD8-PE antibody (CST, USA) were added to stain the lymphocytes in dark for 30 min and washed twice with PBS. The proportion of $CD4^+$T cells and $CD8^+$T cells accounting for $CD3^+$T cells was determined by flow cytometry.

Western blotting and ELISA

The tumor tissues of H22 xenograft mice were collected after twelve days treatment of TB-TF. The expression levels of EGFR, p-EGFR, AKT, p-AKT,

ERK, and p-ERK were determined by western blotting. For cell samples, H22 cells (5×10^5 cells/well) were seeded in 24-well plates. The whole-cell extracts and tumor homogenates were lysed on ice in lysis buffer. Protein concentrations were determined by the BCA protein assay. Equal amounts of protein in each sample were resolved by SDS-PAGE and transferred to PVDF membranes. After blocking, the membranes were incubated overnight at 4 ºC with primary antibodies (anti-EGFR, anti-AKT, anti-ERK1/2, anti-pEGFR, anti-pAKT, and anti-pERK1/2 antibody at 1:1000; CST, USA). After washed with TBST for three times, the membranes were incubated with the secondary antibody (1:5000; CST, USA) for 1 h at room temperature and developed with ECL. The protein levels were analyzed using ImageJ software and normalized to their respective controls. The relative expression of p-EGFR, p-AKT, and p-ERK was presented as normalized ratio of phosphorylated/total protein.

Serum of H22 xenograft mice with or without TB-TF oral administration was collected and IL-6, TNF-α, and IL-17 levels were measured using ELISA kits (Servicebio).

Hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC)

After fixation in 4 % paraformaldehyde, paraffin-embedded tumor and organ tissues were sectioned at 4 μm and stained with hematoxylin-eosin. In addition, IHC was performed on tumor samples. Antigen retrieval process was performed using EDTA antigen repair buffer (pH 9.0). The sections were stained overnight at 4 ºC with anti-Bcl-2, anti-Bax, anti-caspase-3, anti-VEGF,

anti-CD31, anti-TGF-β, anti-CD4, and anti-CD8 antibody (Servicebio). After washed with PBS for three times and stained with HRP (Servicebio) at room temperature for 50 min, positive area will be stained in brown with DAB developer.