Supporting Information

Andrographolide exhibits anti-invasive activity against colon cancer cells via inhibition of MMP2 activity

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Supplementary data

Figure 1S Andrographolide treatment does not directly affect MMP2 activity *in vitro*. The CT26 cells (10⁶) were seeded in the 10-cm dish and incubated for 20 hours. The medium was replaced with phenol red-free DMEM medium for 8 hours. Then, the culture medium of CT26 was transferred to the new plate and incubated with vehicle (0.1% DMSO) or various concentrations (1-100 \( \mu \text{M} \)) of andrographolide. After incubation for another 8 hours, the vehicle- or drug-treated medium was collected and analyzed using gelatin zymography assay.

Figure 2S Andrographolide treatment does not inhibit the NF-\( \kappa \text{B} \) activity. The CT26 cells (2x10⁵) were seeded in the 24 well plates and incubated for 20 hours. The cells were then co-transfected with internal control plasmid beta-gal (0.2 \( \mu \)g), together with reporter plasmid pELAM-1 (1\( \mu \)g) which contains NF-\( \kappa \text{B} \)-binging sites or control plasmid GFP (1 \( \mu \)g), using the FuGENE6 method (Roche, Switzerland). Twenty four hours after transfection, the cells were treated with vehicle (0.1% DMSO) or various concentrations of andrographolide for five hours. Cells were then lysed in lysis buffer (Promega) and assayed for luciferase activity with a luminometer (AutoLumat LB953; Berthold Technologies). The luciferase activity of the vehicle- or drug-treated transfected cells was compared with the activity of untreated cells (blank).