**Scientific Research Process**

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Title: Astragaloside IV inhibits pathological functions of gastric cancer-associated fibroblasts

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1 What did this study explore?

To investigate the inhibitory effects of astragaloside IV on the pathological functions of cancer-associated fibroblasts, and to explore the underlying mechanism.

2 How did the authors perform all experiments?

Paired gastric normal fibroblast (GNF) and gastric cancer-associated fibroblast (GCAF) cultures were established from resected tissues. The GCAFs were treated with vehicle control or different concentrations of astragaloside IV. Conditioned media were prepared from the GNFs, GCAFs, control-treated GCAFs and astragaloside IV-treated GCAFs, and used to culture BGC-823 human gastric cancer cells. Proliferation, migration and invasion capacities of the BGC-823 cells were determined with MTT, wound healing and transwell invasion assays, respectively. The expression of microRNAs in the GCAFs was detected by RT-qPCR. The expression and secretion of the oncogenic factor M-CSF and the tumor suppressive factor TIMP2 in the different groups of GCAFs were determined by western blot and ELISA analysis, respectively. The expression of the oncogenic pluripotency factors SOX2 and NANOG in the BGC-823 cells cultured with different conditioned media was also examined with RT-qPCR and western blot analysis.

3 How did the authors process all experimental data?

All statistical analyses were performed with SPSS 17.0 for Windows (SPSS Inc., USA). To determine the statistical differences between two groups, we used the Student’s *t*-test. To determine the statistical differences between multiple groups, we used the one-way analysis of variance. The significance level was set at *P* <0.05.

4 How did the authors deal with the pre-study hypothesis?

To determine whether astragaloside IV can inhibit the malignancy-promoting capacities of GCAFs, conditioned media from the GNFs, GCAFs, control-treated GCAFs and astragaloside IV-treated GCAFs were used to culture BGC-823 cells, and proliferation, migration and invasion capacities of the BGC-823 cells were measured. To explore the action mechanism of astragaloside IV, the expression of microRNA-214, microRNA-301a, M-CSF and TIMP2 in the GCAFs was detected, and the expression of SOX2 and NANOG in the BGC-823 cells was also detected.

5 What are the novel findings of this study?

Astragaloside IV can inhibit the pathological functions of GCAFs through correcting their dysregulation of microRNA expression; it is promisingly a potent therapeutic agent for gastric cancer patients.