San-Zhong-Kui-Jian-Tang exerts antitumor effects associated with decreased cell proliferation and metastasis by targeting ERK and the epithelial–mesenchymal transition pathway in oral cavity squamous cell carcinoma

Pei-Yu Hsu[1,2], Jiun-Liang Chen[1,3] , Shun-Li Kuo[1,2,3], Wan-Ling Wang[4,5], Fei-Wen Jan[5], Sien-Hung Yang[1,2,3,6*], Chia-Yu Yang[4,5,7,8*]

[1] Department of Traditional Chinese Medicine, Chang Gung Memorial Hospital, Taoyuan, Taiwan

[2] Graduate Institute of Clinical Medical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan

[3] School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan

[4] Molecular Medicine Research Center, Chang Gung University, Taoyuan, Taiwan

[5] Department of Microbiology and Immunology, College of Medicine, Chang Gung University, Taoyuan, Taiwan

[6] Research Center for Chinese Herbal Medicine, Chang Gung University of Science and Technology, Taoyuan, Taiwan

[7] Department of Otolaryngology Head and Neck Surgery, Chang Gung Memorial Hospital, Taoyuan, Taiwan

[8] Graduate Institute of Biomedical Sciences, College of Medicine, Chang Gung University, Taoyuan, Taiwan

* Corresponding author: Chia-Yu Yang and Sien-Hung Yang

Chia-Yu Yang, Department of Microbiology and Immunology, College of Medicine, Chang Gung University, Taoyuan, Taiwan

Address: No.259, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33302, Taiwan (R.O.C.)

Tel:+886-3-2118800#3527 /Fax:+886-3-2118700

Email: chiayu-yang@mail.cgu.edu.tw

Sien-Hung Yang, School of Traditional Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan

Address: No.259, Wenhua 1st Rd., Guishan Dist., Taoyuan City 33302, Taiwan (R.O.C.)

Tel:+886-3-2118800#5101

Email: dryang@mail.cgu.edu.tw

Table S1. Composition of SZKJT

Component	Chinese name	Family	Part used	Origin/Batch number	Weight (g)
Scutellaria baicalensis Georgi	Huang Qin	Labiatae	roots	Shan Xi (PR China)/M1204-1904021	4.0
Gentiana scabra Bunge	Long Dan	Gentianaceae	roots and rhizome	Liao Ning (PR China)/M1705-1902251	2.5
Trichosanthes kirilowii Maxim.	Tian Hua Fen	Cucurbitaceae	roots	Jiang Su (PR China)/M1093-1901071	2.5
Phellodendron chinense C.K.Schneid.	Huang Bo	Rutaceae	bark	Si Chuan (PR China)/M1203-1901181	4.0
Anemarrhena asphodeloides Bunge	Zhi Mu	Liliaceae	rhizome	Nei Meng (PR China)/M0824-1902201	2.5
Platycodon grandiflorus (Jacq.) A.DC.	Jie Geng	Campanulaceae	roots	An Hui (PR China)/M1015-1902151	2.5
Laminaria japonica Aresch.	Kun Bu	Laminariaceae	kelp	Bei Hai Dao (Japan) / M0958-1901181	2.5
Bupleurum chinense DC.	Chai Hu	Umbelliferae	roots	Shan Xi (PR China)/M1055-1904161	2.5
Glycyrrhiza uralensis Fisch.	Gan Cao	Leguminosae	roots and rhizome	Gan Su (PR China)/ M0521-1901031	1.5
Sparganium stoloniferum	San Leng	Sparganiaceae	rhizome	Zhe Jiang (PR China)/M0311-	1.5

(Graebn.) Buch.-Ham. ex Juz				1710181	
Curcuma phaeocaulis Valeton	E Zhu	Zingiberaceae	rhizome	Guang Xi (PR China)/M1110-1805221	1.5
Forsythia suspense (Thunb.) Vahl	Lian Qiao	Oleaceae	fruits	Shan Xi (PR China)/M1132-1901301	1.5
Pueraria lobata (Willd.) Ohwi	Ge Gen	Leguminosae	roots	Si Chuan (PR China)/M1188-1903111	1.5
Paeonia lactiflora Pall.	Bi Shao	Ranunculaceae	roots	An Hui (PR China)/M0502-1904151	1.0
Angelica sinensis (Oliv.) Diels	Dang Gui	Umbelliferae	roots	Gan Su (PR China)/M1307-1708071	1.0
Coptis chinensis Franch.	Huang Lian	Ranunculaceae	rhizome	Si Chuan (PR China)/M1205-1901301	1.0
Cimicifuga dahurica (Turcz.)Maxim.	Sheng Ma	Ranunculaceae	rhizome	Liao Ning (PR China)/M0437-1812241	0.5

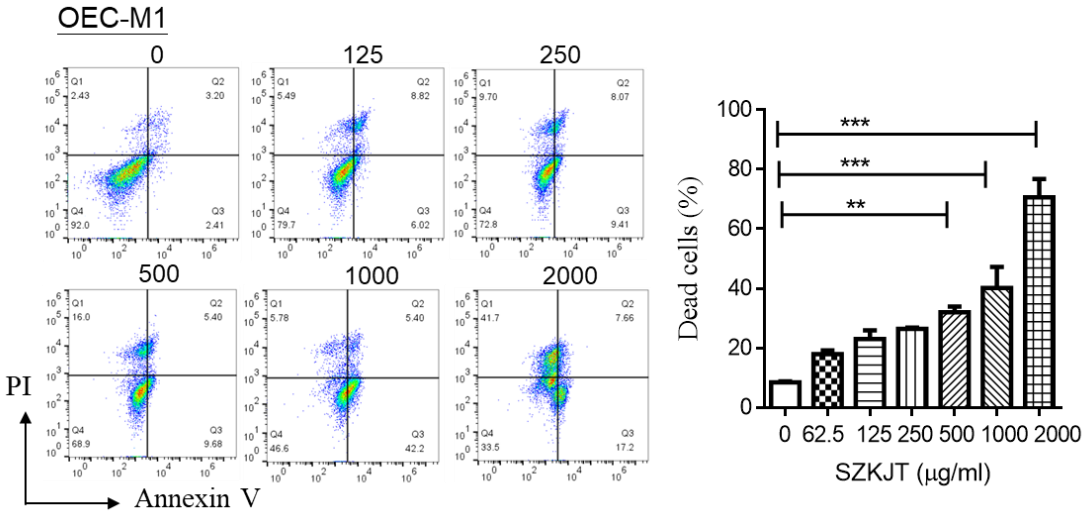

Figure S1. SZKJT induced OEC-M1 cell death. Flow cytometry analysis of OEC-M1 cell treated with a control or SZKJT at different concentrations (125, 250, 500, 1000, or 2000 μg/mL) for 48 h. Quantification of cell death in OEC-M1 cell by Annexin V-FITC/PI staining. The bar diagram shows the percentages of death cells. The results were obtained from three independent experiments. ***$p < 0.001$; **$p < 0.01$.

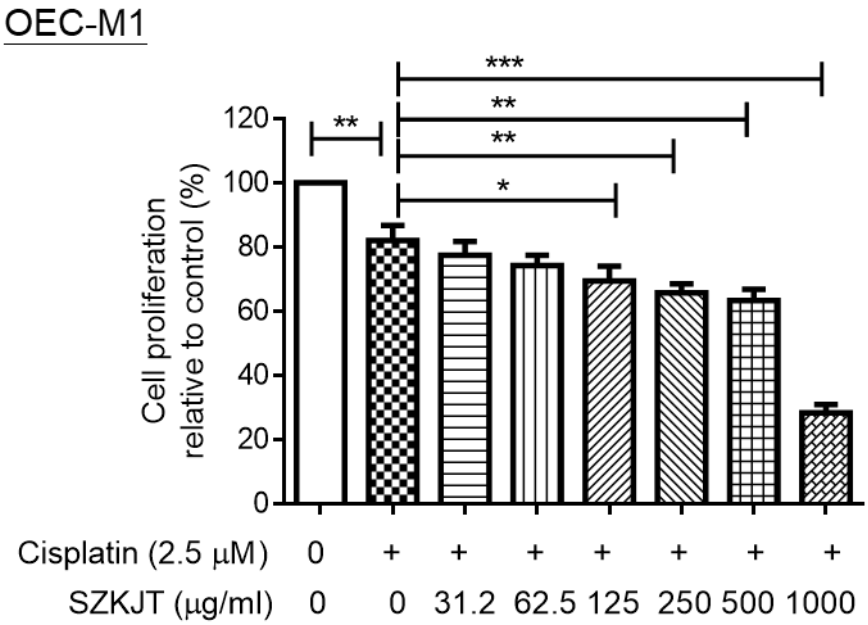

Figure S2. Concurrent treatment with SZKJT enhanced the therapeutic efficacy of cisplatin in OEC-M1 cell line. Cells were treated with a control treatment, 2.5 μM cisplatin, or 2.5 μM cisplatin with and SZKJT at different doses for 48 h and then assessed by MTT assay. The results are expressed as the percent cell proliferation relative to the proliferation of the control. Cisplatin and SZKJT synergistically inhibited proliferation in the OECM1 cell line. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

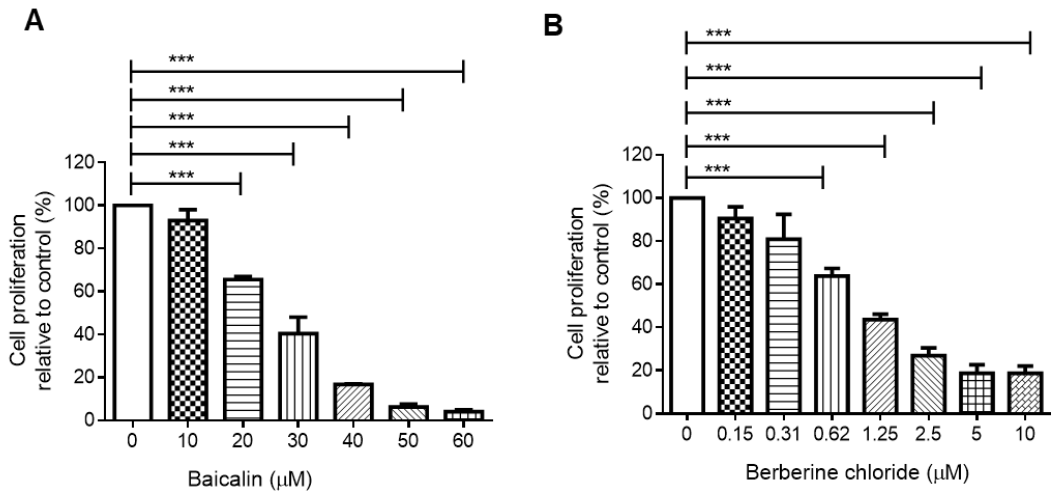

Figure S3. Inhibitory effect of baicalin and berberine on the proliferation of SAS.

SAS cells were treated with a control, baicalin (A), or berberine (B) at different doses for 48 h and assessed by MTT assay. The results are expressed as the percent cell proliferation relative to the proliferation of the control. ***$p < 0.001$.

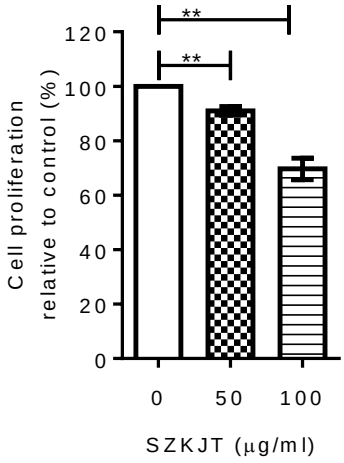

Figure S4. The effect of SZKJT on the proliferation of SG cells. SG cells were treated with SZKJT at different doses for 48 h and then analyzed by MTT assay. The results are expressed as the percent cell proliferation relative to the proliferation of the control. $^{**}p < 0.01$; $^{*}p < 0.05$.