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IGHG1 functions as an oncogene in tongue squamous cell carcinoma *via* JAK1/STAT5 signaling

Y.-L. ZHENG[1], Y.-Y. LI[1], J.-F. XIE[2], H.-Q. MA[1]

[1]Department of Stomatology, Jining No. 1 People's Hospital, Jining, Shand[illegible], China
[2]The Disinfection Supply Center of Jining No. 1 People's Hospital, Jining, S[illegible]ina

Yanliang Zheng and Yunyun Li contributed equally to the study

Abstract. – OBJECTIVE: We explored the IgG1 heavy chain constant region (IGHG1) roles in tongue squamous cell carcinoma (TSCC) progression, as well as to probe the underlying mechanisms.

PATIENTS AND METHODS: The expression patterns of IGHG1 in TSCC tissues and cell lines were tested by Western blotting, quantitative real-time PCR (RT-PCR) and immunohistochemistry (IHC) technologies. The relationship between IGHG1 expression level and the overall survival and clinicopathologic features of patients with TSCC were evaluated to assess the clinical value of IGH[illegible] effects of IGHG1 on cell function were de[illegible] by Cell-Counting Kit-8 (CCK-8), clone fo[illegible]on, flow cytometry and *in vivo* tumor formation a[illegible]s.

RESULTS: The expression of IGHG1 in T[illegible] tissues and cell lines was significantly ele[illegible]ed at both mRNA and protein l[illegible] IGHG1 e[illegible]pression levels closely relate[illegible]ification (*p*=0.008), clinical stage (*p*=[illegible]11), an[illegible]ode metastasis (*p*=0.005) in TSC[illegible]tients. U[illegible]gulation of IGHG1 with lentiviru[illegible]tion [illegible] increased Janus kinase 1 ([illegible] the phosphorylation [illegible]el of sig[illegible]nsducer and activator of transcr[illegible]on 5 (STAT5)[illegible]lition, IGHG1 overexpres[illegible]arkedly enhan[illegible]ll proliferation, clon[illegible] and tumorige[illegible]esis and inhibited cell [illegible]poptos[illegible]ereas these effects were abolished when JA[illegible] downregulated in SCC15 a[illegible]CC4 TSCC cell[illegible]

CON[illegible]SIONS: Collectively, this study reveals [illegible]t IGHG1 functions as an oncogene in TS[illegible]a activ[illegible]g JAK1/STAT5 signaling.

Key Wo[illegible]
[illegible]IGHG1, [illegible]TAT5, [illegible]ability, Apoptosis, Tumori[illegible]

Introduction

[illegible]ongue squamous cell carcinoma (TSCC) is the m[illegible]mon type of oral squamous cell carcinoma (OS[illegible]and is characterized by high proliferation and invasion[1-3] [illegible]dvances have b[illegible]n achieved in the imag[illegible] and th[illegible]tic methods in past decades, the survival rate for pa[illegible]with TSCC still remains low [illegible]occult metasta[illegible]Therefore, identification [illegible] key molecular alterations that contribute [illegible]the progression of TSCC is of importance to find [illegible]ective therape[illegible]argets for TSCC.

[illegible]mmunoglobu[illegible]G (IgG), a crucial factor of the a[illegible]e immun[illegible]ystem, constitutes about 80% of to[illegible]ough it is well known that only B-lympho[illegible] and plasma cells express Ig gene, [illegible]reasing evidence has demonstrated that non-B [illegible] as cancer cells, can also express Ig [illegible]ne[illegible]oth of the heavy chain and the light chain of IgG have been identified to exist in esophageal, colon, prostate, ovarian, breast and liver cancers[8-11]. Although the functions of IgG in carcinogenesis have not yet been fully elucidated, IgG has been verified to promote cancer cell proliferation and invasion[8,12]. In addition, Chen et al[10] demonstrated that IgG expression closely associates with tumor stage in soft tissue tumors. However, the knowledge of IgG roles in TSCC is limited.

The Janus kinase (JAK)/signal transducer and activator of transcription (STAT) (JSK/STAT) signaling pathway not only regulates cell proliferation, survival, cycle and differentiation, but also modulates immune responses through acting on multiple cytokines, such as growth factors and hormones[13-15]. Once ligand binds to its cognate receptor, conformational changes will induce receptor oligomerization and the activation of JAKs, which then provides the docking sites for STATs. Subsequently, STATs phosphorylate, dimerize, and translocate to cell nucleus and promote the transcription of target genes[16]. The deregulation of JSK/STAT signaling pathway frequently occurred in OSCC and strongly implicated in OSCC progression[17,18], suggesting a potential of JAK/STAT signaling as a therapeutic target for TSCC treatment.

Corresponding Author: Haiqing Ma, MD; e-mail: ma13853769725@163.com

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In this study, we aimed to investigate the IgG1 heavy chain constant region (IGHG1) roles in TSCC progression, as well as to probe the role of JAK/STAT signaling in IGHG1-invloved TSCC progression.

Patients and Methods

Patients

With the approval of Ethical Committee of Jining No. 1 People's Hospital and the Helsinki Declaration, 105 paired TSCC tissues and the adjacent normal tissues were obtained from patients with TSCC between January 2012 and January 2018. The clinicopathologic characteristics, including gender, age, T classification, clinical stage, node metastasis and differentiation and the overall survival after surgery were all obtained from the electronic medical records. All patients underwent surgery prior to chemoradiotherapy and signed the informed consent.

Immunohistochemistry (IHC) Assay

IHC assay was used to assess IGHG1 p[illegible]in expression in paired TSCC tissues and no[illegible] tissues based on the following pressures. F[illegible] the tissues were paraffin-embedded and cut into [illegible]m sections. Second, the sections were dewaxed, [illegible]drated and incubated with 3% H_2O[illegible] followed [illegible] antigen repairing with Tris-E[illegible]d incubation with 5% goat serum. Th[illegible] the s[illegible]ns were probed with anti-IGHG1 a[illegible]body (1:[illegible] dilution; No. sc-515946, Santa C[illegible]otech[illegible]ta Cruz, CA, USA) o[illegible]erni[illegible]. Next, [illegible]e sections were incub[illegible]d with [illegible]responding secondary antibo[illegible]3, 3'-diamino[illegible]line tetrachloride (Sig[illegible]ch, St. Louis, [illegible], USA) was served a[illegible] sub[illegible] Harris' hematoxylin solution was used to stain [illegible]ucleus.

The st[illegible]ing of IGHG1 i[illegible]ue tissue samples w[illegible] evaluated by two pa[illegible]ologists on the base [illegible] the posi[illegible]ive staining proportion and the sta[illegible] inten[illegible]. The positive staining percentage wa[illegible]s follow[illegible] for ≤5%, 1 for 6-25%, 2 for 26-[illegible] for 51[illegible]75%, and 4 for >75%, re[illegible]ely. In[illegible]s marked as follows: 0 repres[illegible] no stain[illegible] 1 represents weak staining, 2 re[illegible]sents moderate staining, and 3 represents str[illegible] respectively. The final score was [illegible] multiplying the percentage score and [illegible]sity score. A staining score of IGHG1 >6 was co[illegible]ed as IGHG1 high expression and ≤ 6 was cons[illegible]ed as low expression.

Cell Culture

Human normal gingival epitheli[illegible] (ATCC® PCS-200-014™; used as a c[illegible]ol cell [illegible] for TSCC cells), together with TS[illegible] cell lines, including SCC9, SCC15, SCC25 a[illegible]C4 were purchased from American Type C[illegible] Collection (ATCC, Manassas, VA, U[illegible]A). No[illegible]ngival epithelial cells were cultu[illegible] in the mixe[illegible] containing 1/2 Dermal [illegible] Basal Medium ([illegible] PCS-200-030™) and [illegible] Keratin[illegible]te Growth Kit (ATCC® PCS-200-0[illegible] SC[illegible], SCC15 [illegible]CC25 and SCC4 cells w[illegible]re gro[illegible]ulbecco'[illegible]odified Eagle's Mediu[illegible]am's F12 [illegible] (T[illegible]mo Fisher Scientific [illegible]ltham, MA, U[illegible]pplemented with 10% [illegible]e serum (FBS, [illegible]hermo Fisher Scientifi[illegible] Waltha[illegible], USA) and 1% penicillin/streptomycin (Therm[illegible]r Scientific, Waltham, MA [illegible]) at 37 °C with [illegible]O_2.

[illegible]ll Transfection and Treatment

The lentivir[illegible]ectors used to upregulate or [illegible]nregulate IG[illegible]1 in human TSCC cells were ca[illegible] OE-IGH[illegible] or sh-IGHG1 and purchased from [illegible]a (Shanghai, China), as well as their nega[illegible] control vectors (NC). The shRNAs [illegible]d to silence JAK1 and the sh-NC vector (No. [illegible]) were obtained from OriGene (Beijing, [illegible]ina). For cell infection, the aforesaid vectors were added into cell culture medium for 24 hours, followed by replacement with fresh medium and incubation at 37°C for another 24 hours. To construct the stable transfection cell lines, the infected cells were incubated with 5 mg/ml puromycin and/or 100 mg/ml G418 for 14 days.

Quantitative Real-Time PCR (RT-PCR) Analysis

Total RNA was extracted from tissues and cells using the RNApure Tissue & Cell Kit (DNase I) referring to the manufacturer's description (CWBIO, Beijing, China). Then, RNA quantification was carried out on an ND-1000 NanoDrop Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA). After that, the RNA samples were submitted to cDNA reverse transcription and RT-PCR using SuperRT One Step RT-PCR Kit (CWBIO, Beijing, China) on Bio-Rad detection system (Bio-Rad, Hercules, CA, USA). GAPDH level is used to normalize the mRNA level of IGHG1. Primers targeting IGHG1 and GAPDH were obtained from Invitrogen (Carlsbad, CA, USA) and were listed as follows:

IGHG1-Forward: 5'-GCAGCCGGAGAACAACTACA-3', IGHG1-Reverse: 5'-TGGTTGT-

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GCAGAGCCTCATG-3'; GAPDH-Forward: 5'-CACTAGGCGCTCACTGTTCTCTC-3',

GAPDH-Reverse: 5'- GACCAAATCCGTTGACTCCGA-3'.

Western Blotting Assay

Total protein samples extracted from tissues and cells were obtained using radio immunoprecipitation assay (RIPA) lysis buffer containing protease inhibitor. After quantification with a bicinchoninic acid assay (BCA) Protein Assay (Bio-Rad, Hercules, CA, USA), 20-30 mg proteins from each sample were loaded and separated by 10% polyacrylamide gels through electrophoresis. Then, the proteins were transferred to polyvinylidene difluoride membranes (PVDF; Millipore, Billerica, MA, USA). Subsequently, the membranes were incubated with 5% non-fat milk and probed overnight at 4°C with the primary antibodies, including IGHG1 (1:1000 dilution; No. sc-515946, Santa Cruz Biotechnology, Santa Cruz, CA, USA), JAK1 (1:2000 dilution; No. #3332, Cell Signaling Technology, Danvers, MA, USA), JAK2 (1:2000 dilution; No. #3230, Cell Signaling Technology, Danvers, MA, USA), JAK3 (1:2000 dilution; No. #3775, Cell Signaling Technology, Danvers, MA, USA), Tyk2 (1:1000 dilution; No.#9312, Cell Signaling Technology, Danvers, MA, USA), p-STAT5 (1:1000 dilution; No. 9351, Cell Signaling Technology, Danvers, MA, USA), STAT5 (1:1000 dilution; No. #25656, Cell Signaling Technology, Danvers, MA, USA) and GAPDH (1:5000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA). [illegible] incubation with the corresponding secondary antibodies (Santa Cruz Biotechnology), the protein expression in the Western blots was examined on a Western blot imaging and quantitative system (Bio-Rad, Hercules, CA, USA) with the help of enhanced chemiluminescence (ECL) reagent (Millipore, Billerica, MA, USA) and quantified by ImageJ software (National Institutes of Health, Bethesda, MD, USA) after background subtraction.

Immunofluorescence Technology

After being [illegible] transfection with OE-NC or OE-IGHG1, SCC[illegible] and SCC15 cells were fixed in 4% paraformaldehyde for 15 min and then penetra[illegible] Triton X-100 for 20 min. Then, the cells were blocked with 5% non-fat milk for [illegible] at room temperature, followed by incubation with anti-STAT5 antibody (No. #25656, Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. Subsequently, the cells were incubated with Alexa Fluor 488-labeled [illegible] antibodies (Invitrogen, Carlsbad, CA, USA) [illegible] 1 h at room temperature and washed with phosphate-buffered saline (PBS). Di[illegible]ine phenylindoles (DAPI) counterstain was [illegible] stain the nuclei. Stained cells were visualized [illegible] immunofluorescence microscope.

Cell Counting Kit-8 (CCK-8) Assay

To assess cell proliferation, TSCC cells were first inoculated [illegible] 96-well plates at a density of 3×10^3 cells/well and cultured at 37°C overnight. Then, the cells were given di[illegible] infections, including [illegible], sh-NC, OE-IGHG1, OE-NC, sh-JAK1, OE-IGHG1 + sh-JAK1. Subsequently, cell culture medium [illegible] each well was replaced with [illegible] of CCK-8 reagent (Beyotime, Beijing, China) and 90 ml fresh medium. The cells were incubated at 37°C for further 4 hours. The absorbance at 450 nm was measured with a plate reader (Model 680; Bio-Rad, Hercules, CA, USA) every 2[illegible] hours for 6 times.

Flow Cytometry Assay

The effect of IGHG1/JAK1 axis on TSCC [illegible]sis was detected by using Annexin V [illegible]ITC/Propidium Iodide (PI) apoptosis detection kit (KeyGEN Biotech, Jiangsu, China) and measured on flow cytometry (BD Biosciences, San Jose, CA, USA) according to previous study[20].

Clone Formation Assay

TSCC cells were stably transfected with sh-IGHG1, sh-NC, OE-IGHG1, OE-NC, sh-JAK1 or OE-IGHG1 + sh-JAK1. Then, the cells were collected and seeded on a 6-cm dish at a density of 200-300 cells/well. After 14 days incubation at 37°C, cells were stained with 0.1% crystal violet (Solarbio, Beijing, China) for 20 minutes and washed with PBS twice. Clone number was counted under a microscope.

Animal Experiment

The animal experiment was carried out according to the National Institute of Health's Guidelines for the Care and Use of Laboratory Animals and was given permission by the Animal Care and Research Committee of Jining No .1 People's Hospital. Four-week male BALB/c athymic nude mice (Beijing Vital River Laboratory Animal Technology, Beijing, China) were fed in specific pathogen free conditions. To construct the tumor-bearing mouse models, 5×10^6

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SCC4 cells with stable transfection of sh-JAK1, OE-IGHG1, OE-IGHG1 + sh-JAK1 or their controls were injected subcutaneously into the nude mice. After 4 weeks of cell transplantation, the mice were euthanized and tumors were weighted.

Statistical Analysis

Data were obtained from as least three independent experiments and expressed as mean ± standard deviation (SD). SPSS 22.0 software (SPSS Inc., IBM, Armonk, NY, USA) was used to perform data analysis. The relationship between IGHG1 expression levels and the clinicopathologic feature was assessed by the chi square test (χ^2-test). Data conformed to Gaussian distribution was analyzed by the *t*-test and one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test. $p<0.05$ was considered to be statistically significant.

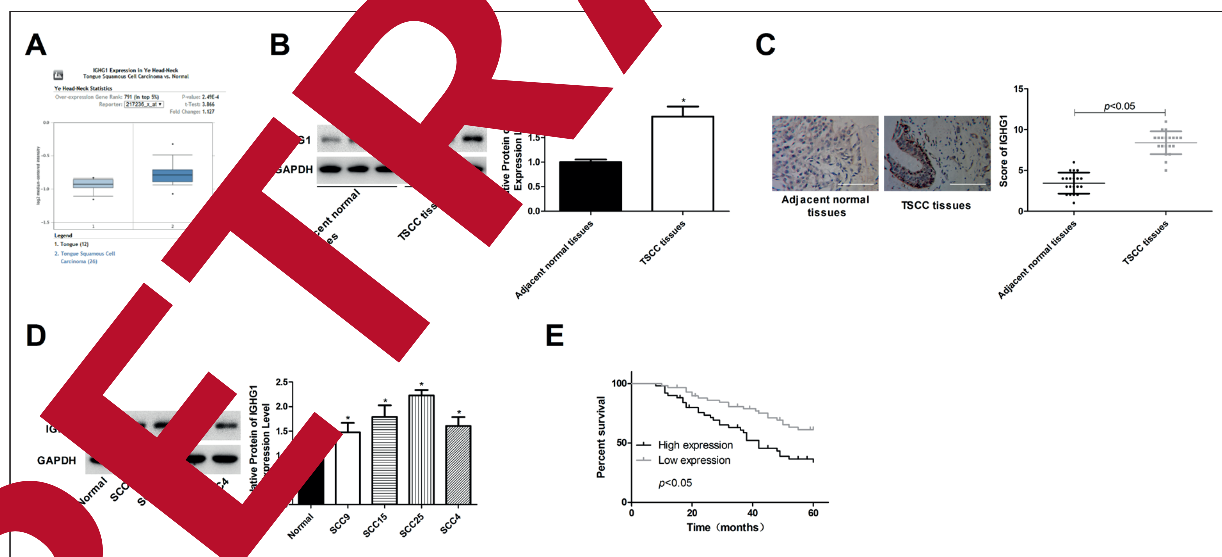
Results

IGHG1 Expression Was Elevated in TSCC Tissues and Cells

First, we assessed the expression patte[illegible] IGHG1 in TSCC tissues and cells. Acc[illegible]g the oncomine database, which included [illegible]ses of normal tongue samples and 26 cases of [illegible]C samples, we found that the expression of IG[illegible] was significantly increased in TSCC as compared with that of the normal tongues (Fig[illegible] further confirm the high expressi[illegible] pattern [illegible] IGHG1 in TSCC tissues, we [illegible] performed Western blotting and IHC ass[illegible] As shown in Figure 1B-1C, it was easy to se[illegible]GHG1 expression was significantly [illegible]levated [illegible]C tissues in comparison with [illegible] adjacent n[illegible] tissues. Consistently, the [illegible]otein level of IG[illegible] TSCC cell lines, incl[illegible]ng SCC[illegible]CC15, SCC25 and SCC4 was notab[illegible]her [illegible]n that of [illegible]e normal tongue cell l[illegible]e (Fig[illegible]. These [illegible]ve discoveries sugg[illegible]d that the [illegible] exp[illegible]ion level of IGHG1 m[illegible] take part in t[illegible]rrence and developm[illegible]C.

High Expression o[illegible]HG1 Related to Adv[illegible]d Clinical F[illegible]s and Shorter O[illegible] Survival in Patients with TSCC

To uncover the role of IGHG1 in the progres[illegible]n of TSCC, w[illegible]en evaluated the clinical val[illegible]f IGHG1 in [illegible]CC. Fifty-five patients with I[illegible] high ex[illegible]ssion (IHC score >6) and 50 patie[illegible]G1 low expression (IHC score ≤6) were chosen for this study. Compared with [illegible] low expression group, patients in the high [illegible] group showed higher T classification [illegible]), clinical stage ($p=0.011$), and node metastasis ($p=0.005$), with no evident difference



Fig[illegible]eased expression of IGHG1 was detected in TSCC tissues and cells. **A**, Oncomine database showed that IG[illegible] in TSCC samples (n=26) was significantly higher than that of the normal tongue samples (n=12). The ex[illegible]sion of IGHG1 protein in TSCC tissues and the adjacent normal tissues were determined by using (**B**) western blotting and [illegible]C technologies (scale bar = 80 m; x100). **D**, Western blotting analysis of the protein level of IGHG1 in normal gingival ep[illegible]ell line (Normal) and TSCC cell lines (SCC4, SCC15, SCC9 and SCC25). **E**, The relationship between IGHG1 expres[illegible]n levels and the overall survival rates in patients with TSCC was determined by survival curve analysis. (*$p<0.05$).

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Table I. Evaluation of the relationship between IGHG1 expression levels and the clinicopathologic features of patients with TSCC.

Characteristics	High expression	Low expression	*p*-value
Gender			0.332
Male	23	26	
Female	32	24	
Age (years)			0.235
<60	31	34	
≥60	24	16	
T classification			0.0[illegible]
T1-T2	29	39	
T3-T4	26	11	
Clinical stage			0.0[illegible]
I-II	22	33	
III-IV	33	17	
Node metastasis			0.005
No	25	37	
Yes	30	13	
Differentiation			0.[illegible]
Well	20	24	
Moderate	25	21	
Poor	10	[illegible]	

in the gender, age and differentiation (Table I). Moreover, the overall survival rate in IGHG1 high expression group was significantly lower th[illegible] of the low expression group (Figure 1E[illegible] results demonstrated IGHG1 played a sig[illegible]ant role in predicting the prognosis and clinica[illegible]-cess of TSCC.

IGHG1 Promoted Cell Pro[illegible]n and Repressed Cell Apo[illegible]sis [illegible]CC

We carried out CCK-[illegible]nd flow [illegible]tometry assays to assess IGHG1 [illegible] in TS[illegible]sion. The mRNA an[illegible] prot[illegible]ssion [illegible]s of IGHG1 in SCC1[illegible]nd SCC[illegible]s were significantly increa[illegible] when cells [illegible]infected with OE-IGH[illegible]eas IGHG1 [illegible]pression was reduced [illegible]en th[illegible]s were infected with sh-2 (Figu[illegible] 2A-2B). As [illegible]howing the highest knoc[illegible]wn efficiency a[illegible]he 3 shRNAs, we cho[illegible]sh-2 for following ex[illegible]eriments. CCK-8 res[illegible] showe[illegible] that OE-IGHG1 infection signif[illegible] enh[illegible]ed cell growth, and sh-IGHG1 infect[illegible]ened cel[illegible]rowth in both SCC15 (Figure 2[illegible] SCC[illegible]ell lines (Figure 2D). In [illegible]n, OE[illegible]gnificantly enhanced cell clo[illegible]rmation a[illegible]y, and sh-IGHG1 induced an opp[illegible]e result in both SCC15 and SCC4 cell lines (Fi[illegible]reover, OE-IGHG1 inhibited cell [illegible]ptosis, [illegible] sh-IGHG1 induced cell apoptosis, [illegible]etected by the flow cytometry assay (Figu[illegible] These results demonstrated that IGHG1 serv[illegible]s an oncogene in TSCC.

I[illegible]1 Promo[illegible]d the Activation of J[illegible] Signaling in TSCC Cells

To disclose the mechanisms underlying IG[illegible]G1-mediated TSCC development, we investi[illegible]ther JAK/STAT signaling pathways [illegible]ere [illegible]olved. Among all members of JAK fami[illegible]y, only the expression of JAK1 was markedly upregulated when SCC15 and SCC4 cells were infected with OE-IGHG1 (Figure 3A and 3C). Then, we assessed the expression and phosphorylation levels of STAT5 protein, the downstream of JAK1 pathway. The results showed that the phosphorylation level of STAT5 was apparently increased when IGHG1 was overexpressed in SCC15 and SCC4 cells (Figure 3B and 3D). Moreover, IGHG1 upregulation significantly facilitated the nuclear accumulation of STAT5 protein (Figure 4). These results demonstrated that IGHG1 promoted the activation of JAK1/STAT5 signaling in TSCC cells.

Inhibition of JAK1/STAT5 Signaling Abolished IGHG1 Roles in Promoting TSCC Progression

To further clarify JAK1/STAT5 roles in IGHG1-mediated TSCC progression, we then recruited sh-JAK1 to knock down JAK1 expression in SCC15 and SCC4 cell lines. The expressions of JAK1 at both mRNA and protein levels were all significantly reduced when cells were infected with the sh-2 targeting JAK1 (Figure 5A-5B). Besides, the enhancements in cell proliferation (Figure 5C-5D) and clone

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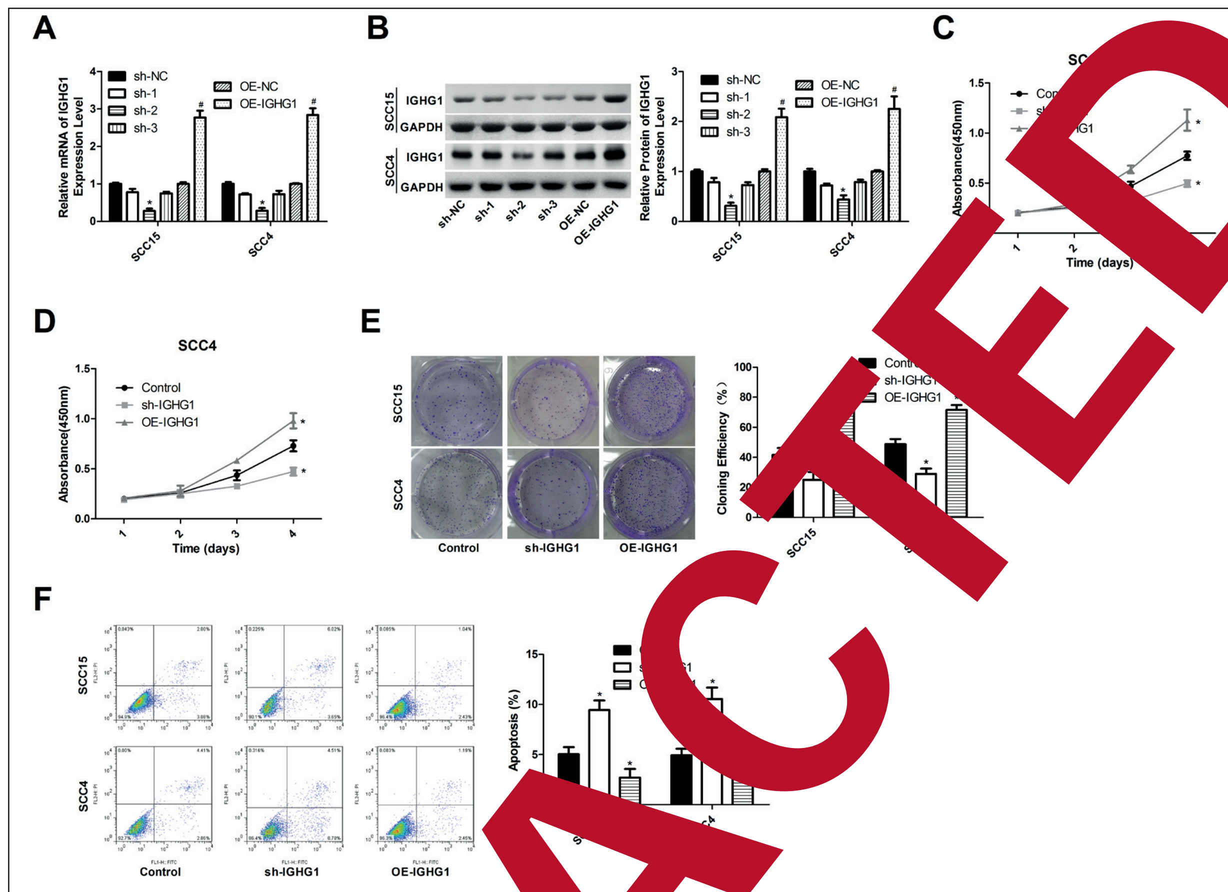

Figure 2. Evaluation of IGHG1 effects on cell growth, [illegible]rmation and apoptosis in TSCC cells. SCC4 and SCC15 cells were infected with OE-IGHG1, sh-IGH[illegible]their contro[illegible]rs, then, the infected efficiencies were determined by (**A**) RT-PCR and (**B**) Western blotting assay[illegible]rowth wa[illegible]sed by CCK-8 assay. **E**, The clone formation ability of SCC4 and SCC15 cells were determined [illegible]lone fo[illegible]ion assay. [illegible]l apoptosis was tested by flow cytometry assay. (*p<0.05).

formation (Figure5E) [illegible]d re[illegible]cell apopto-sis (Figure 5F) indu[illegible] by IGH[illegible]rexpression were all rescued w[illegible]JAK1 was do[illegible]lated in SCC15 and SC[illegible] Moreover, IGH[illegible] overex-pression in SC[illegible] cells [illegible]cantly enhanced cell *in vivo* tumor formation abi[illegible]d deletion of JAK1 inhibited [illegible]or growth and in[illegible] IGHG1 role in promoti[illegible] tumor formation (Figu[illegible]6). These results sugg[illegible]d that I[illegible]HG1 promoted the progression of TS[illegible]ough[illegible]vating JAK1/STAT5 signaling.

[illegible]ussion

I[illegible] is a glycoprotein generated by mature B cel[illegible] cells to recognize and neutral-[illegible]various [illegible]thogens/antigens[21]. IgG expresses [illegible]any kinds of human cancers, such as breast, p[illegible] lung, colon, thyroid, esophagus, pla-centa[illegible]ophoblast and sarcomas[8,10,22-24]. Notably, IgG is importance of carcinogenesis. Blockade of IgG with either antisense RNA or IgG antibody could significantly suppress cancer cell growth and induce cell apoptosis in several kinds of cancers, including colorectal cancer[25], prostate cancer[11], and urothelial carcinoma[26]. Although Khurram et al[27] has reported that the serum con-tent of IgG4 was increased in OSCC, the role of IgG in TSCC still needs to be further clarified. In the present study, we demonstrated, for first time, that the expression of IGHG1 was signifi-cantly increased in TSCC tissues and cells, and its high expression predicted a poor prognosis and advanced clinical status in patients with TSCC, indicating that IGHG1 might play an im-portant role in the occurrence and development of TSCC. Consistently, IGHG1 has been detected by the antigen of COC166-9 antibody in ovari-an cancer and its high expression correlates with poor prognosis[28].

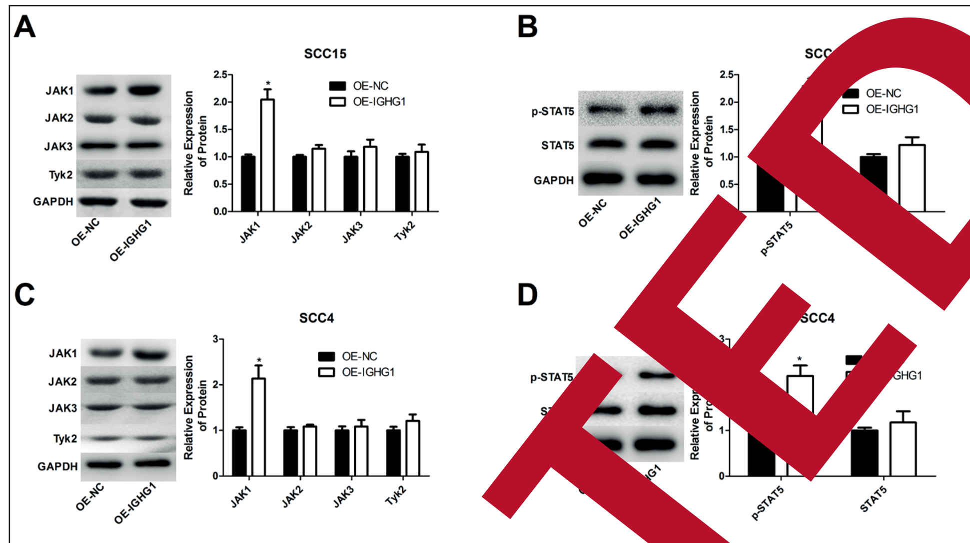

Figure 3. Upregulation of IGHG1 promoted the activation of JAK1/STAT5 in TSCC cells. SCC4 and SCC15 cells were transiently infected with OE-NC or OE-IGHG1, then, the western blotting analysis was performed to detect the protein expression levels of JAK1, JAK2, JAK3, Tyk2, p-STAT5 and STAT5 in (**A-B**) SCC15 cells and (**C-D**) SCC4 cells. (*p<0.05).

The mechanisms contributing to tumor cell survival are complex, involving many signaling pathways regulating cell proliferation, apoptosis and differentiation[29]. On the basis of this, we performed the gain-/loss-of-function assays to explore IGHG1 roles in TSCC progressi... r the first time. We observed that upregul... IGHG1 significantly promoted cell proli...on, clone formation and *in vivo* tumor formatio...l-ities and repressed cell apoptosis in both S... and SCC15 cell lines, suggesting that IGH... functions an oncogene in TSC...esults a... consistent with previous stu... in o... cancers. So, Pan et al[11] and Xu et al[3]...ported t... siRNA–mediated downregulatio... GHG1... dent inhibition in cell viability and an increase ... cell apoptosi... prostate cancer. Li et al[31] re-...ed that block... of IGHG1 could significantly i... cell *in v*... tumor formation ability and imp... val rate of tumor-bearing mice in pancreatic cancer.

Although it has been found that cancer cells ...ss IgG[8,10,22-24], the molecular mecha-...sms ... which IgG facilitates tumor progression remain undiscovered. So far, several hypotheses have been put forward. Wang et al[32] revealed that the production of reactive oxygen species (ROS) induced by IgG promoted tumor cell growth and proliferation. They also identified that ROS scavengers induced a significant repression in cancer

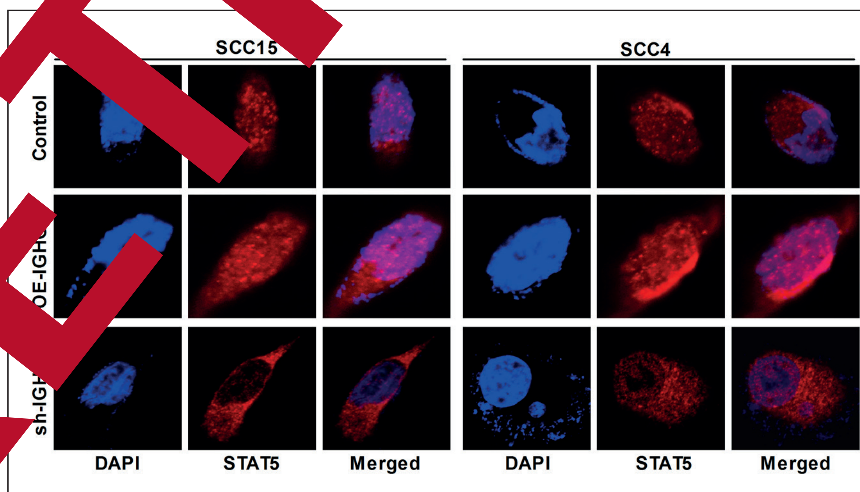

Fig... Upregulation of IGHG1 promoted the nuclear accumulation of STAT5 in TSCC cells. Following cell infection, immun...luorescence was carried out to assess the subcellular location of IGHG1 protein (x600). (*p<0.05).

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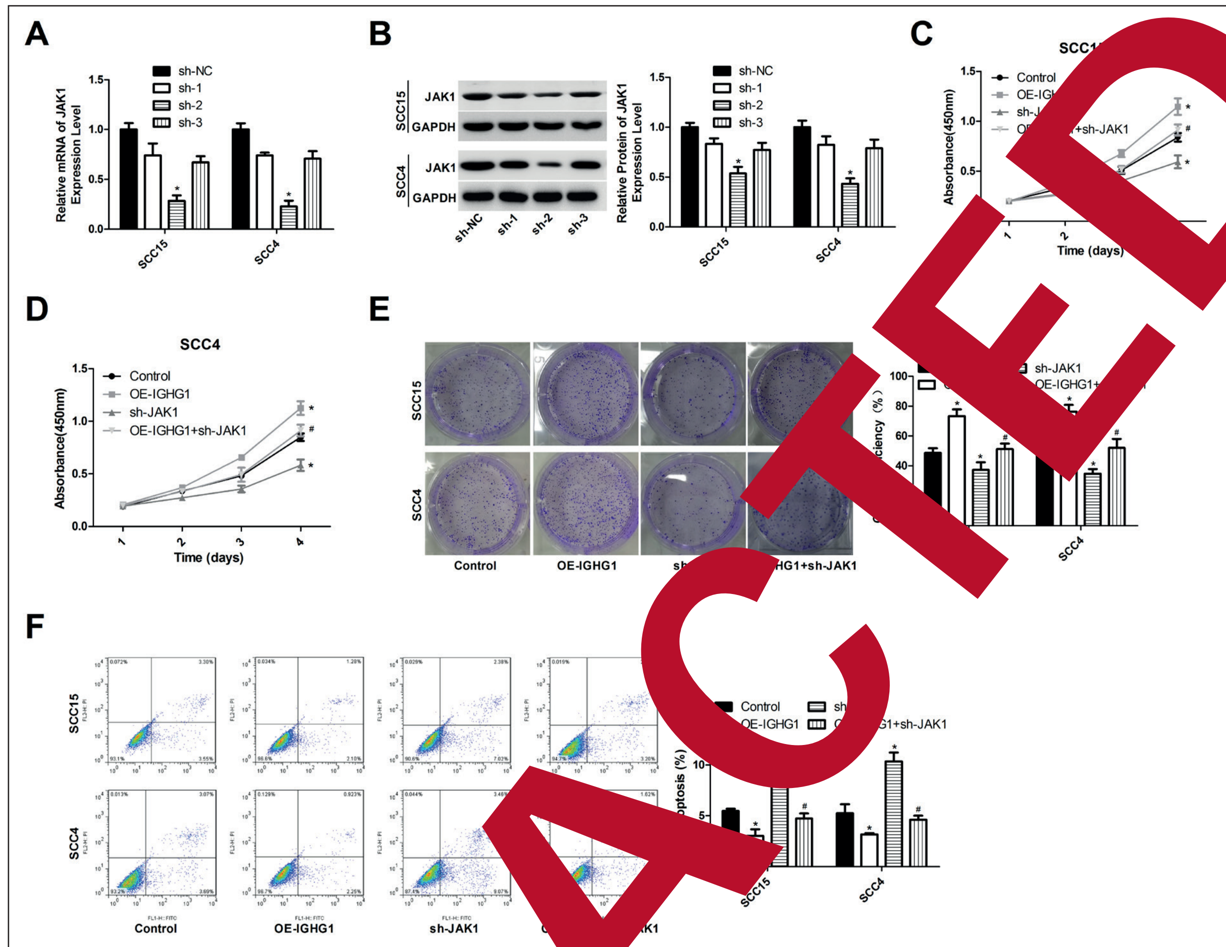

Figure 5. Knockdown of IGHG1 i[illegible]G1 roles [illegible]moting cell viability and inhibiting cell apoptosis in TSCC cells. **A-B**, The knockdown efficie[illegible]s of sh[illegible]G1 was ass[illegible] by RT-PCR and western blotting assays. **C-D**, Cell viability was determined by CCK-8 assa[illegible]SCC4 and [illegible]C15 cells. **E**, [illegible]one formation assay was used to assess cell viability in SCC4 and SCC15 cells. **F**, The effec[illegible]GHG1/JA[illegible] cell apoptosis were determined in SCC4 and SCC15 cells. (*$p<0.05$, compared with control group; #[illegible]om[illegible]IGHG1 group).

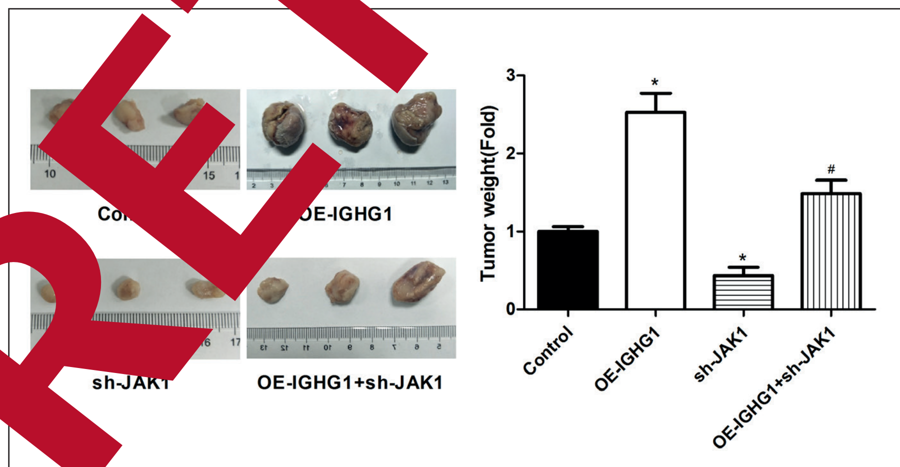

Figure 6. IGHG1 promoted cell in vivo tumor formation ability through activating JAK1 signaling. *In vivo* tumor-bearing experiments were performed to assess cell tumorigenesis in differently stable transfection SCC4 cell lines. (*$p<0.05$, compared with control group; #$p<0.05$, compared with OE-IGHG1 group)

cell growth in IgG-deficient cancer cells via inhibiting the mitogen-activated protein kinase/extracellular-regulated kinase (MAPK/ERK) pathway. Wang et al[33] demonstrated that IgG promoted cell growth through lipopolysaccharide (LPS)/Toll-like receptor 4 (TLR4)-mediated inflammatory responses in cervical cancer. In the current study, we demonstrated a new mechanism by which IGHG1 accelerated the progression of TSCC. We observed that the expression of JAK1 and phosphorylation level of STAT5 were notably increased when SCC4 and SCC15 cells were transfected with OE-IGHG1, suggesting that IGHG1 can promote the activation of JAK1/STAT5 signaling. In addition, we found that inhibition of JAK1/STAT5 signaling via sh-JAK1 neutralized IGHG1 effects on cell viability and tumorigenesis promotions and cell apoptosis inhibition, indicating that IGHG1 promotes TSCC progression via activating the JAK1/STAT5 signaling.

Furthermore, some evidence has suggested that the oncogenic roles of IgG due to its role in the immune system. Qiu et al[22] found that IgG protected cancer cells from host immune responses through interacting with complement prot[illegible] to form the immune complexes in papillary [illegible] cancer. Moreover, high levels of serum I[illegible] can whittle natural kill cell-mediated antibo[illegible]pendent cell cytotoxicity in antibody-based t[illegible]apy[34,35], suggesting that IgG may promote canc[illegible] progression via modulating th[illegible] respons[illegible]es. Notably, JAK1 has also [illegible] pr[illegible]d to be involved in immune resp[illegible]es[13], sugg[illegible]ing that IGHG1 may promote TS[illegible] progress[illegible] [illegible]AK1/STAT5 mediate[illegible] dere[illegible] [illegible] system. However, t[illegible] opinion [illegible] to be clarified in our furthe[illegible]dies.

Concl[illegible]s

The [illegible]rrent investigation re[illegible]als that IGHG1 funct[illegible]s as an [illegible]cogene in TSCC via activating the [illegible]/STA[illegible] signaling, which might be a potent ta[illegible]SCC tre[illegible]ent.

Au[illegible]s' Contri[illegible]ons

Y.Z.[illegible]. and J.X. designed experiments, analyzed data and [illegible]lts. Y.Z., Y.L. and J.X. analyzed and i[illegible]ient data. Y.Z. and Y.L. data acquisition. [illegible] Y.L. and J.X. wrote the manuscript and prepared [illegible]res. Y.Z., Y.L. and H.M. reviewed and edited the ma[illegible]. H.M. coordinated and directed the project. All autho[illegible]pproved the final version of the manuscript.

Ethics Approval and Consent to Pa[illegible]

Experiments involving human samples w[illegible]pproved [illegible] the Ethical Committee of Jining No. 1 [illegible]ple's Hospital and the Helsinki Declaration. Writte[illegible]formed consent was obtained from all participant[illegible] experiments. The animal experiment was carried o[illegible]ing to the National Institute of Health's G[illegible]delines [illegible]re and Use of Laboratory Animals a[illegible]was given p[illegible] b[illegible] Animal Care and Research [illegible]mmittee of Jini[illegible] People's Hospital.

Conflict of Int[illegible]sts

The authors de[illegible] that they have [illegible] of interests.

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