Int. J. Morphol.,
40(6):1587-1593, 2022.

RGD Peptide Inhibits Tumor Growth by Affecting Angiogenesis Related Signaling Pathway of Laryngeal Cancer Stem Cells

El Péptido RGD Inhibe el Crecimiento Tumoral al Afectar la Vía de Señalización Relacionada con la Angiogénesis de las Células Madre del Cáncer de Laringe

Xudong Wei[1]; Rui Lv[1]; Xinyi Wang[2]; Jiayi He[1] & Jian He[1]

WEI, X.; LV, R.; WANG, X.; HE, J. & HE, J. RGD peptide inhibits tumor growth by affecting angiogenesis related signaling pathway of laryngeal cancer stem cells. *Int. J. Morphol., 40(6)*:1587-1593, 2022.

SUMMARY: This study is to investigate the role and mechanism of RGD peptide in laryngeal cancer stem cells (CSCs). Laryngeal cancer $CD133^+$Hep-2 CSCs were sorted by flow cytometry. RGD peptide was co-cultured with sorted laryngeal CSCs. Cell proliferation was detected with CCK-8 assay. The mRNA levels of VEGF/VEGFR2/STAT 3/HIF-1α were detected with RT-PCR. The proteins of VEGF/VEGFR2/STAT 3/HIF-1α were detected with Western blot. The sorted CSCs were inoculated into nude mice. Tumor volume was measured. Integrin αvβ3 expression in tumor tissues was analyzed with immunohistochemistry. The results showed that the ratio of $CD133^+$ CSCs to the total number of cells was 1.34±0.87 %, while $CD133^-$non-tumor stem cells accounted for 95.0±5.76 %. The sorted cancer stem cells grew well. The RGD peptide significantly inhibited the proliferation of $CD133^+$Hep-2 laryngeal CSCs in a dose-dependent manner. The RGD peptide significantly inhibited the mRNA of VEGFR2, STAT3 and HIF-1α in laryngeal CSCs in a concentration-dependent manner. Consistently, the RGD peptide significantly inhibited the protein expression of VEGFR2, STAT3 and HIF-1α in laryngeal CSCs in a dose-dependent manner. At the same time, *in vivo* tumor experiments showed that the RGD peptide significantly inhibited tumor volume but not the body weight. Furthermore, RGD peptide significantly inhibited the expression of tumor angiogenesis-related protein integrin αvβ3. Our findings demonstrate that RGD peptide inhibits tumor cell proliferation and tumor growth. The underlying mechanism may that RGD inhibits tumor angiogenesis-related signaling pathways, thus affecting the tumor angiogenesis, and decreasing the progression of human laryngeal CSCs.

KEY WORDS: RGD peptide; Integrin αvβ3; $CD133^+$ stem cells; VEGF; Tumor growth.

INTRODUCTION

Laryngeal cancer is the second most common malignant tumor of the respiratory tract after lung cancer (Global Burden of Disease Cancer Collaboration *et al*., 2015). At present, the main treatment method for laryngeal cancer is surgery. However, radiotherapy or chemotherapy resistance and postoperative recurrence and metastasis are the main causes of laryngeal cancer-related death. Therefore, there is an urgent need to find effective treatment strategies.

Cancer stem cells (CSCs) are cells in malignant tumors that have unlimited proliferation, self-renewal and multi-differentiation capacities. They are considered to be the source of tumor cell proliferation and metastasis (Clevers, 2011; Yu *et al*., 2012). It has been shown by our group and other scholars that laryngeal cancer has heterogeneity in differentiation, proliferation, tumorigenicity, and resistance to radiotherapy and chemotherapy, and there are CSCs in laryngeal cancer (Prince *et al*., 2007; Wei *et al*., 2009). These CSCs may lead to recurrence and metastasis of laryngeal cancer (Li & Zhang, 2014). Therefore, to find effective targets for CSCs in laryngeal cancer is the key for precision treatment of laryngeal cancer (Maccalli *et al*., 2014; Deshmukh *et al*., 2016; Ahmad & Amiji, 2017). It is reported that in the tumor microenvironment, CSCs and angiogenesis are inseparable, and are related to tumor formation and development (Yu *et al*., 2007). As a glycosylated protein, human CD133 has 2 extracellular loops and 5 transmembrane domains (Miraglia *et al*., 1997; Grosse-Gehling *et al*., 2013).

[1] Department of Otolaryngology Head and Neck Surgery, Gansu Provincial Hospital, Lanzhou 730000, Gansu, PR China.
[2] Department of Otolaryngology Head and Neck Surgery, General Global China Railway Xian Hospital, Xi'an, 710054, Shanxi, PR China.

FUNDING. This work was supported by National Natural Science Foundation of China (No. 81860475, Natural Science Foundation of Gansu Province (20JR10RA377), Lanzhou Talent Innovation and Entrepreneurship Project (2021-RC-129), Gansu Excellent Young Talents Project in Health Field (GSWSQN2021-002)

Received: 2022-08-15 Accepted: 2022-10-13

It is one CSC marker in Hep-2 cells (Zhou *et al*., 2007). One *in vivo* study showed that Hep-2 cell line with positive CD133 expression had higher tumorigenic potential than those with negative CD133 expression (Wei *et al*., 2009). In this study, we used CD133 to mark CSCs in laryngeal cancer.

Integrins are a family of transmembrane receptors that facilitate cell-extracellular matrix adhesion. They are highly expressed on the surface of neovascular endothelial cells and various tumor cells, and play an important role in tumor angiogenesis, migration and infiltration (Sun *et al*., 2014). They have two subunits of a and b. Integrin αvβ3 plays an important role in tumor invasion and metastasis, and has the ability to recruit and activate matrix metalloprotein-2 and plasmin, which degrades matrix membrane and matrix interstitial components and promotes tumor metastasis (Huveneers *et al*., 2007). Studies have shown that integrin αvβ3 is highly expressed in $CD133^+$ laryngeal CSCs (Lu *et al*., 2011; Li *et al*., 2013).

RGD peptide is a widely distributed tripeptide of arginine-glycine-aspartate (Arg-Gly-Asp). It is the recognition site for integrin αvβ3 and its ligand, which mediates the specific binding of matrix proteins to integrin αvβ3, thereby regulating the cell-cell interaction and cell-extracellular matrix interaction (Ahmedah *et al*., 2017; Katsamakas *et al*., 2017) Studies have shown that RGD peptide can directly activate caspase-3 and cause tumor cell apoptosis. Exogenous RGD peptides can competitively bind to integrin αvβ3 ligand and reduce the expression of integrin αvβ3, thereby inhibiting tumor angiogenesis and reducing the adhesion and infiltration ability of tumor cells (Semenza, 2007; Cook *et al*., 2009: Shin *et al*., 2015).

Although the role of RGD peptide in inhibiting tumor angiogenesis has been widely recognized, its effect on CSCs and tumor angiogenesis of laryngeal cancer is unclear. Here, in this study, we investigated the inhibitory effect of RGD peptide on laryngeal CSCs, and the expression of tumor angiogenesis-related molecules including VEGF, VEGFR2, STAT3 and HIF-1α. The possible mechanisms were analyzed and discussed. Our study will provide a new perspective for laryngeal cancer treatment.

MATERIAL AND METHOD

Animals. Nude mice (4-6-week-old; 28-32s body weight) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. They were kept in standard conditions. This study was conducted in strict accordance with the recommendations of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals. The research protocol with animal experimentation was approved by the Ethics Committee of Gansu Provincial Hospital (Protocol Number: Not applicable).

Cell culture. Human laryngeal carcinoma Hep-2 cells were from Qi Biotechnology Co., Ltd. (Shanghai, China). They were cultured in RPMI 1640 medium (Hyclone, Utah, Logan City, USA) containing 10 % fetal bovine serum (Clark, USA) and 1 % penicillin/streptomycin (Gibco, Grand Island, New York City, USA) in a 37 °C, 5 % CO_2 cell incubator.

Fluorescence activated cell sorting. FITC-anti-human CD 133 (BIOSS, Beijing, China) was added to the single cell suspension and incubated for 30 min. After washing, samples were analyzed using a Beckman Coulter MoFlo XDP to sort $CD133^+$ CSCs. The cell purity was measured with flow cytometry.

CCK-8 assay. $CD133^+$Hep-2 laryngeal cancer stem cells were seeded in 96-well plates and cultured for 4 h. CCK-8 reagent (Dojindo, Japan) was then added and the incubation continued for 2.5 h. The absorbance value OD450 of each well was measured and the standard curve was fitted.

The sorted $CD133^+$Hep-2 laryngeal CSCs were seeded in 96-well plates at 10^3 cells/well. After culture for 24 h, 10 μL RGD peptide (Abcam, England) at different concentrations (14.4 μM, 144 μM, 288 μM, 576 μM, and 1440 μM) were added and incubated for 24h. Finally, 10 μL of CCK-8 from the CCK-8 kit was added to each well. After incubation for 2.5 h, the absorbance of each well at 450 nm was measured by a microplate reader.

The cell survival rate was calculated according to the standard curve and according to the following formula: cell survival rate = [(As-Ab)/(Ac-Ab)]× 100 %. As: experimental well (cells, CCK-8, different concentrations of RGD peptide); Ac: control well (cells, CCK-8, no RGD peptide); Ab: blank well (without cells, RGD peptides, and CCK-8).

RT-PCR. The sorted $CD133^+$Hep-2 laryngeal CSCs were seeded at 5×10^5 in T25 flasks and incubated with different concentrations of RGD peptide (10 μM, 20 μM, 40 μM, respectively). After 24 h, the cells were collected and lysed with RNAiso Plus (TaKaRa, Japan). Total RNA was extracted from cells using an RNeasy kit (QIAGEN, Germany). The cDNA was obtained with SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, USA). The primer sequences were shown in Table I. The PCR Master Mix (SYBR Green; QIAGEN) and the Real-Time PCR System (7500 model; ABI) was used. b-actin was used as internal control.

Table I. RT-PCR primers.

Gene	5'-3' Forward Primer	5'-3' Reverse Primer
VEGF	CCTTGCTGCTCTACCTCCAC	AGCTGCGCTGATAGACATCC
VEGFR2	GATCTGAAACGGCGCTTGGA	TTCGCGATGCCAAGAACTCC
STAT3	GGCCATCTTGAGCACTAAGC	CGGACTGGATCTGGGTCTTA
HIF-1_	CCACCTATGACCTGCTTGGT	TTCATATCCAGGCTGTGTCG
Beta-actin	ATACTCCAAACCTTTCCACC	AGTTTTCCTTGGGGTCCAGA

Western blot. The sorted CD133$^+$Hep-2 laryngeal CSCs were seeded at 5 x 10^5 in T25 flasks and incubated with different concentrations of RGD peptide (10 μM, 20 μM, 40 μM, respectively). After 24 h, the cells were harvested and total proteins were extracted. After SDS-PAGE, proteins were transferred to PVDF membranes (Millipore, Bedford, MA). The primary antibodies were rabbit anti mouse anti-VEGF (Proteintech), anti-VEGFR2 (Proteintech), anti-STAT3 (Proteintech), anti-HIF-1α (Proteintech) and β-actin (ZSGB-BIO). The goat anti-rabbit secondary antibodies were from ZSGB-BIO. Enhanced chemiluminescence kit (Pierce Rockford, IL) was used for color development. The grayscale of the western blot protein band was analyzed by Image.

Tumor growth. The mice were randomly divided into four groups, with 10 mice in each group. Before drug administration, mice in two groups received right axillary subcutaneous injection of CD133$^+$ CSCs (3×10^5), and those in the other two groups received right axillary subcutaneous injection of CD133$^-$ tumor cells (3×10^5). When the tumor diameter reached 0.5-1 cm, the mice in CD133$^+$ group and CD133$^-$ group were further divided into PBS control group and 10 mg/g/d RGD peptide group, respectively. RGD peptide was administrated every other day. The administration lasted for 30 days. Mouse body weight and tumor length and width were recorded every other day. Tumor volume was calculated according to the following formula: tumor volume = tumor length × tumor width^2 × 0.52. At the end of the experiment, mice were sacrificed and the tumors were dissected.

Immunohistochemistry. The dissected tumor tissues were fixed with 10 % paraformaldehyde for 12 h at room temperature. Then, the tissue was dehydrated with ethanol, embedded in paraffin and cut into sections of 0.5 μm. The primary antibody of integrin αvβ3 (Santa Cruz, Mexico) was added and incubated overnight at 4 °C. After washing, the sections were incubated with the secondary antibody (SP-9002; ZSGB-BIO, China) for 15 min at room temperature. Finally, the sections were stained with chrome solution for 10 min, counter stained with hematoxylin for 20 min, and mounted with neutral resin. The sections were observed under optical microscope. Integral Optical Density (IOD) was calculated using Imagepro plus software (Media Cybernetics, Maryland, USA).

Statistical analysis. The statistical data was analyzed and processed by IMB SPSS16.0. All data are expressed as mean ± standard error of mean (mean ± SEM). Multiple comparisons were performed using one-way ANOVA followed by LSD (Least Significant Difference) (for variables with homogeneity of variance) or rank sum test (for variables without homogeneity of variance). A $P<0.05$ was considered as statistically significant.

RESULTS

Sorting of Hep-2 CD133$^+$ CSCs. In this experiment, laryngeal cancer CD133$^+$Hep-2 CSCs were sorted by flow cytometry. The results showed that the ratio of CD133$^+$ CSCs to the total number of cells was 1.34±0.87 %, while CD133- non-tumor stem cells accounted for 95.0±5.76 %. The sorted cancer stem cells grew well.

RGD peptide toxicity by CCK-8 assay. The effect of different concentrations of RGD peptide (14.4 μM, 144 μM, 288 μM, 576 μM, 1440 μM) on the proliferation of CD133$^+$ CSCs was examined using CCK-8 assay. The IC50 (half maximal inhibitory concentration) of RGD was determined as 29.11 μM. As shown in Figure 1A and 1B, RGD peptide inhibited the proliferation of Hep-2 CD133$^+$ cells in a dose-dependent manner. These results indicate that the RGD peptide has a significant inhibitory effect on the proliferation of laryngeal CSCs.

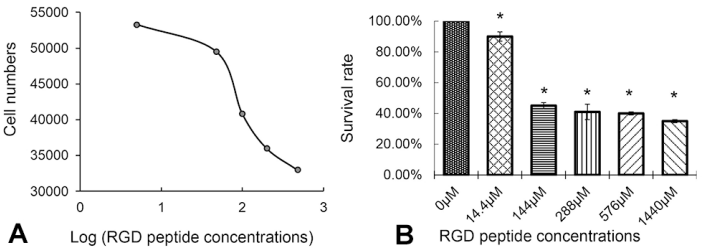

Fig. 1. Effect of RGD peptide on cell proliferation. CD133$^+$ CSCs of laryngeal cancer were treated with RGD at different concentrations as indicated. (A) The cell number was shown. (B) The cell survival rate was shown. Compared with 0 mM, *$p<0.05$.

Inhibition of RGD peptide on VEGF pathway in laryngeal CSCs. To examine the effect of RGD peptide on VEGF pathways and downstream effectors in laryngeal CSCs, RT-PCR was performed to determine the mRNA levels of related genes. The results showed that the mRNA levels of VEGF, VEGFR2, STAT3 and HIF-1α were significantly decreased in a dose-dependent manner after co-culture of RGD peptide with laryngeal CSCs for 24 h (Fig. 2).

To further verify this, Western blot was performed. As shown in Figure 3, similar to mRNA result, the expression levels of VEGF, VEGFR2, STAT3 and HIF-1α protein decreased gradually as the increase of RGD peptide concentration. These results indicate that the RGD peptide inhibits the expression of key genes involved in VEGF pathway.

Inhibition of tumor growth by RGD peptide. To investigate the effect of RGD peptide on tumor formation of $CD133^+$ Hep-2 cancer cells, we inoculated $CD133^+$ tumor stem cells into nude mice. The CD133- Hep-2 cells were used as controls. The gross tumor volume in $CD133^-$ +PBS group was smaller than $CD133^+$ +PBS group while that in mice treated with RGD peptide was smaller than that in PBS control (Fig. 4A). Statistically, compared with $CD133^-$ +PBS group, the tumor volume of $CD133^+$ +PBS group was significantly larger ($P<0.05$) (Fig. 4B & 4D), suggesting that $CD133^+$ Hep-2 cancer cells are more tumorigenic than $CD133^-$ Hep-2 cells *in vivo*, and have the characteristics of stem cells. After RGD treatment, the tumor volume was significantly decreased ($P<0.05$), indicating that RGD peptide significantly inhibits tumor growth. Moreover, the body weight of nude mice treated with RGD peptide was significantly higher than that of the PBS control group, indicating that the mice are in good condition and that RGD peptide has no obvious toxicity to nude mouse at IC50 dose (Fig. 4C). The above results demonstrate that RGD can inhibit the growth of tumor stem cells in nude mice.

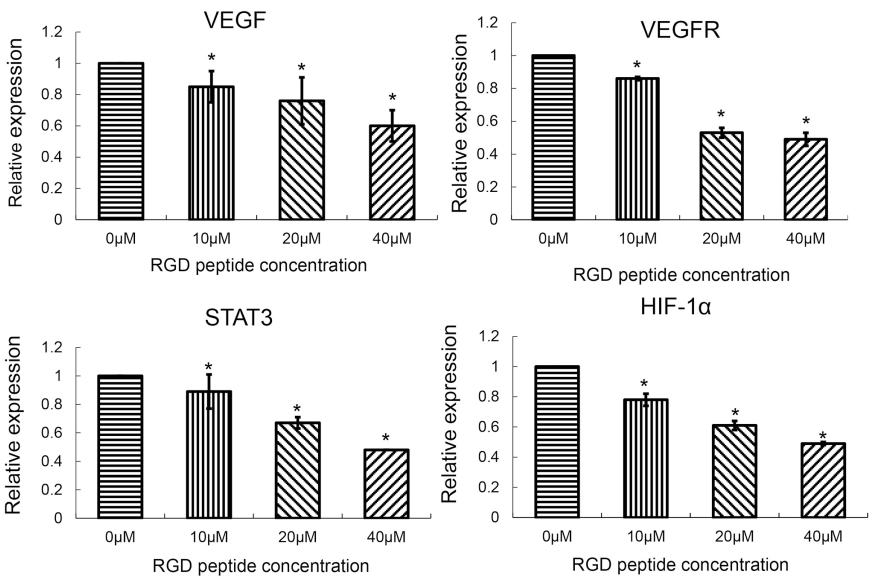

Fig. 2. Effect of RGD peptide on the mRNA expression of genes in VEGF pathway. The mRNA expression level of VEGF, VEGFR2, STAT3 and HIF-1α were respectively analyzed by RT-qPCR after treatment with RGD peptide for 24 h. *p < 0.05, ** p < 0.01.

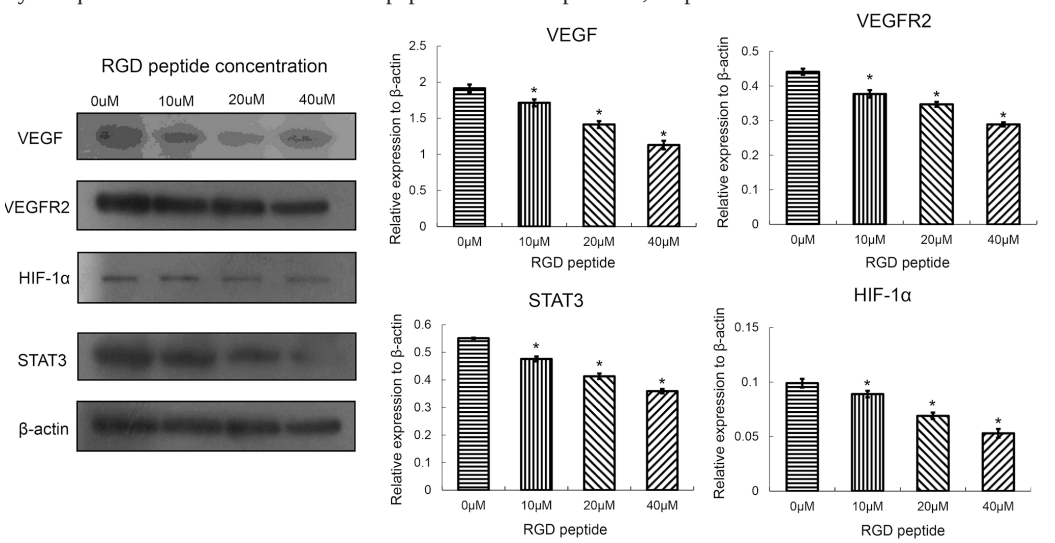

Fig. 3. Effect of RGD peptide on the protein expression of genes in VEGF pathway. The protein expression level of VEGF, VEGFR2, STAT3 and HIF-1a were analyzed by Western blot after treatment with RGD peptide for 24h. Representative and quantitative Western blot results were shown. *p < 0.05, ** p < 0.01.

Effect of RGD peptide on integrin αvβ3. To detect the effect of RGD peptide on integrin αvβ3 expression, immunohistochemistry was performed. The integrin avb3 was stained brown. It was mainly expressed on the cell membrane and some was in cell nucleus. It was observed that RGD peptide can significantly reduce the expression of integrin αvβ3 in CD133$^+$ Hep-2 CSCs (IOD was respectively 11.33±0.65, 110.54±4.58, P<0.001) (Fig. 5). Meanwhile, the RGD peptide was also able to significantly reduce the level of CD133$^-$Hep-2 integrin αvβ3 (IOD was respectively 13.64±0.69, 53.56±3.31, P<0.001). These results showed that RGD inhibited tumor vascular endothelial integrin αvβ3 expression, suggesting that tumor neovascularization is inhibited, thus inhibiting tumor growth.

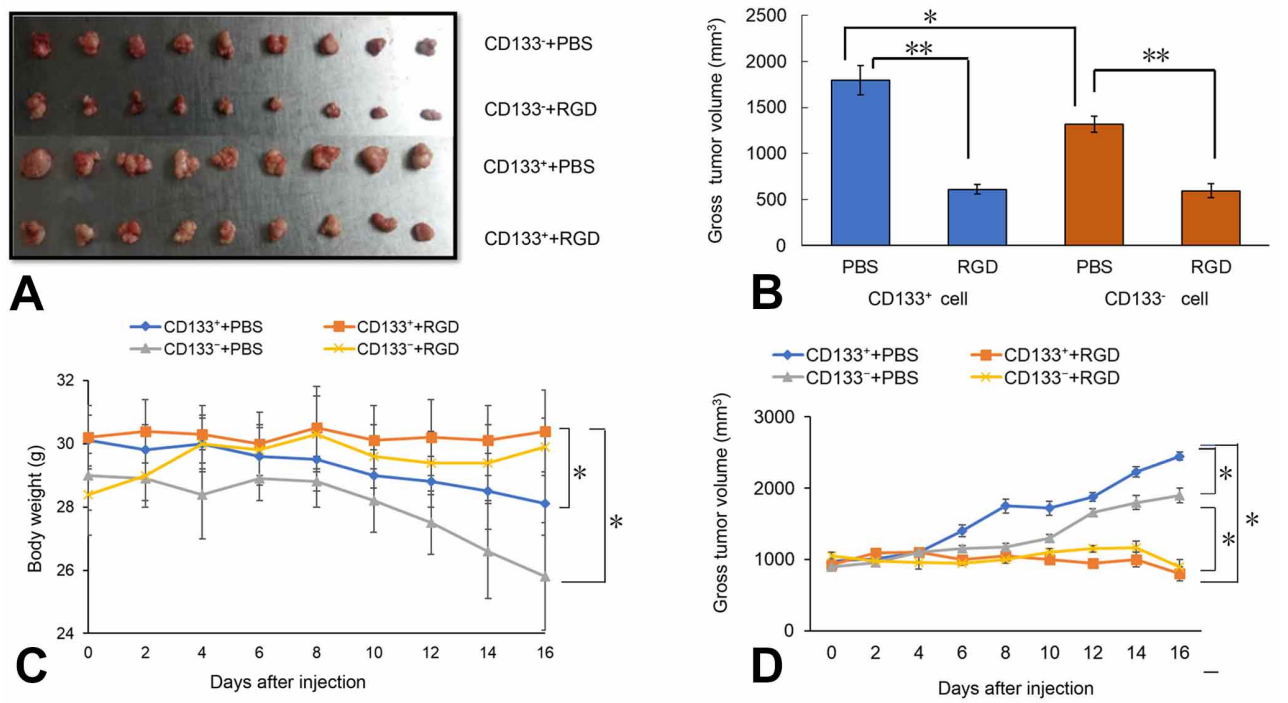

Fig. 4. Effect of RGD peptide on tumor growth *in vivo*. Nude mice were inoculated with tumor cells (CD133$^+$ CSCs or CD133$^-$ CSCs) and then treated with RGD peptide. Tumor volume and body weight were measured. (A) Photograph of removed tumors was shown. (B) The tumor volume in each group on day 10 was statistically analyzed. (C) The body weight of mice was comparatively analyzed. (D) The tumor volume changed with the time of administration. *p < 0.05, ** p < 0.01.

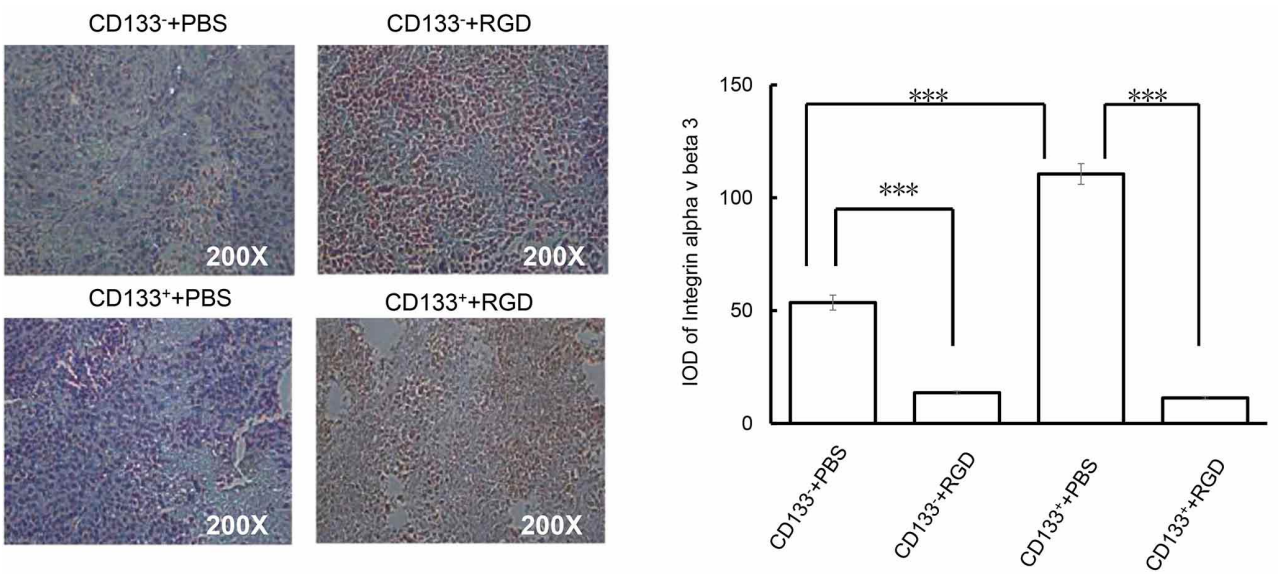

Fig. 5. Effect of RGD peptide on integrin αvβ3 expression. Immunohistochemistry was performed to detect integrin αvβ3 expression in tumor tissues. The integral optical density (IOD) of integrin αvβ3 was comparatively analyzed and shown. ***p < 0.001.

DISCUSSION

Laryngeal cancer is one of the most common malignant tumors of the head and neck (Global Burden of Disease Cancer Collaboration *et al*., 2015). Although the 5-year survival rate and quality of life of patients have been improved, the recurrence and metastasis of laryngeal cancer after treatment are still the most important cause of death. So far, there is still a lack of effective treatments to prevent and treat tumor recurrence and metastasis. In recent years, CSC theory has provided a new perspective for the research and treatment of tumors (Greco *et al*., 2016; Wang *et al*., 2017). Zhou *et al*. (2007) showed that although $CD133^+$ cells accounted for only 3.15 %±0.83 % of the total number of laryngeal cancer cells, they had the characteristics of stem cells.

Tumor growth depends on its angiogenesis. VEGF is an important signaling molecule involved in tumor angiogenesis (Ferrara & Henzel, 1989; Kim *et al*., 2002). It binds to VEGF receptor (VEGFR), thereby activating multiple signaling pathways, promoting cancer cell proliferation, migration and invasion, and angiogenesis (Rafii *et al*., 2002). VEGFR is a single transmembrane receptor protein, including VEGFR1 (Fit-1), VEGFR2 (KDR) and VEGFR3 (Fit-4), all of which belong to Protein Tyrosine Kinase Receptor (PTKR) and participate in angiogenesis. Among them, VEGFR2 is mainly involved in the process of angiogenesis (Zhang *et al*., 2010). Signal transducers and activators of transcription 3 (STAT 3) is a transcription factor (Darnell Jr. *et al*., 1994) and is highly expressed in a variety of malignancies (Germain & Frank, 2007; Al Zaid Siddiquee & Turkson, 2008). Moreover, STAT3 plays an important pivotal role in mediating signaling between tumor cells and other cells (Yu *et al*., 2007). Zhou *et al*. (2016) found that persistent activation of STAT3 was associated with the development of laryngeal cancer. HIF-1 is a transcription factor produced under hypoxia. When activated, HIF-1 can promote tumor angiogenesis. It is a heterodimer consisting of HIF-1α and HIF-1β. HIF-1β, also known as ARNT (aryl hydrocarbon receptor nuclear translocator), is stably expressed in cells, and its gene is located in human Chromosome q21 region. HIF-1a localizes in q21-24 region of human chromosome 14, and is regulated by an anoxic signal (Pugh & Ratcliffe, 2003). Thus, VEGF/VEGFR2/STAT3, and HIF-1α signaling pathway, synergistically promotes tumor angiogenesis (Chen *et al*., 2008; Xu *et al*., 2012; Wang *et al*., 2017).

Integrin αvβ3 plays an important role in tumor proliferation, adhesion and angiogenesis. It is highly expressed in endothelial cells of tumor neovessels and on tumor cell surface (Sun *et al*., 2014). In the extracellular matrix, the RGD peptide competitively inhibits the binding of integrin αvβ3 to the receptor, thereby attenuating integrin-mediated tumor cell adhesion. In this study, we sorted $CD133^+$ Hep-2 CSCs and co-cultured with RGD peptides. We found that RGD peptides inhibited tumor proliferation. Meanwhile, the mRNA and protein levels of VEGF/VEGFR2/STAT3 signaling pathway were decreased by RGD peptide, indicating that RGD peptide may inhibit $CD133^+$ Hep-2 cancer cells by inhibiting VEGFR2-mediated STAT 3/HIF-1α pathway-related genes and protein expression. Furthermore, we observed the inhibitory effect of RGD peptide on tumor growth in nude mice, and detected the expression of tumor angiogenesis-related protein integrin αvβ3. The results showed that the expression level of αvβ3 in the RGD peptide treatment group was significantly lower than that in the PBS control group.

CONCLUSION

In conclusion, RGD peptide may inhibit tumor growth by inhibiting the proliferation of $CD133^+$ Hep-2 cancer cells. The underlying mechanism is that RGD inhibits tumor angiogenesis-related signaling pathways, thus affecting the tumor angiogenesis, and decreasing the progression of human laryngeal cancer stem cells.

ACKNOWLEDGEMENTS . We are grateful to the Lanzhou Branch of the Chinese Academy of Sciences for their technical support. In addition, we sincerely thank Wang Jingyu of the Basic Medical College of Lanzhou University for the guidance and help.

WEI, X.; LV, R.; WANG, X.; HE, J. & HE, J. El péptido RGD inhibe el crecimiento tumoral al afectar la vía de señalización relacionada con la angiogénesis de las células madre del cáncer de laringe. *Int. J. Morphol., 40(6)*:1587-1593, 2022.

RESUMEN: Este estudio se realizó para investigar el papel y el mecanismo del péptido RGD en las células madre del cáncer de laringe (CSC). Las CSC $CD133^+$Hep-2 de cáncer de laringe se clasificaron mediante citometría de flujo. El péptido RGD se cocultivó con CSC laríngeas clasificadas. La proliferación celular se detectó con el ensayo CCK-8. Los niveles de ARNm de VEGF/VEGFR2/STAT 3/HIF-1α se detectaron con RT-PCR. Las proteínas de VEGF/VEGFR2/STAT 3/HIF-1α se detectaron con Western blot. Las CSC clasificadas se inocularon en ratones nudos. Se midió el volumen del tumor. La expresión de integrina αvβ3 en tejidos tumorales se analizó con inmunohistoquímica. Los resultados mostraron que la proporción de CSC $CD133^+$ con respecto al número total de células fue de 1,34 ± 0,87 %, mientras que las células madre no tumorales CD133 representaron el 95,0 ± 5,76 %. Las células madre cancerosas clasificadas crecieron bien. El péptido RGD inhibió significativamente la proliferación de CSC laríngeas $CD133^+$Hep-2 de una manera dependiente de la dosis. El péptido RGD inhibió significativamente el ARNm de VEGFR2, STAT3 y HIF-1α en CSC laríngeas de manera dependiente de la concentración. De manera consistente, el péptido RGD

inhibió significativamente la expresión proteica de VEGFR2, STAT3 y HIF-1α en CSC laríngeas, de manera dependiente de la dosis. Al mismo tiempo, los experimentos con tumores *in vivo* mostraron que el péptido RGD inhibía significativamente el volumen del tumor pero no el peso corporal. Además, el péptido RGD inhibió significativamente la expresión de la proteína integrina αvβ3 relacionada con la angiogénesis tumoral. Nuestros hallazgos demuestran que el péptido RGD inhibe la proliferación de células tumorales y el crecimiento tumoral. El mecanismo subyacente puede ser que RGD inhiba las vías de señalización relacionadas con la angiogénesis tumoral, afectando así la angiogénesis tumoral y disminuyendo la progresión de las CSC laríngeas humanas.

PALABRAS CLAVE: Péptido RGP; Integrina αvβ3; Células madre CD133⁺; VEGF, crecimiento tumoral.

REFERENCES

Ahmad, G. & Amiji, M. M. Cancer stem cell-targeted therapeutics and delivery strategies. Expert Opin. Drug Deliv., 14(8):997-1008, 2017.

Ahmedah, H. T.; Patterson, L. H.; Shnyder, S. D. & Sheldrake, H. M. RGD-binding integrins in head and neck cancers. *Cancers (Basel), 9(6)*:56, 2017.

Al Zaid Siddiquee, K. & Turkson, J. STAT3 as a target for inducing apoptosis in solid and hematological tumors. *Cell Res., 18(2)*:254-67, 2008.

Chen, S. H.; Murphy, D. A.; Lassoued, W.; Thurston, G.; Feldman, M. D. & Lee, W. M. F. Activated STAT3 is a mediator and biomarker of VEGF endothelial activation. *Cancer Biol. Ther., 7(12)*:1994-2003, 2008.

Clevers, H. The cancer stem cell: premises, promises and challenges. *Nat. Med., 17(3)*:313-9, 2011.

Cook, K. M.; Hilton, S. T.; Mecinovic, J.; Motherwell, W. B.; Figg, W. D. & Schofield, C. J. Epidithiodiketopiperazines block the interaction between hypoxia-inducible factor-1alpha (HIF-1alpha) and p300 by a zinc ejection mechanism. *J. Biol. Chem., 284(39)*:26831-8, 2009.

Darnell Jr., J. E.; Kerr, I. M. & Stark, G. R. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science, 264(5164)*:1415-21, 1994.

Deshmukh, A.; Deshpande, K.; Arfuso, F.; Newsholme, P. & Dharmarajan, A. Cancer stem cell metabolism: a potential target for cancer therapy. *Mol. Cancer, 15(1)*:69, 2016.

Ferrara, N. & Henzel, W. J. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. *Biochem. Biophys. Res. Commun., 161(2)*:851-8, 1989.

Germain, D. & Frank, D. A. Targeting the cytoplasmic and nuclear functions of signal transducers and activators of transcription 3 for cancer therapy. *Clin. Cancer Res., 13(19)*:5665-9, 2007.

Global Burden of Disease Cancer Collaboration; Fitzmaurice, C.; Dicker, D.; Pain, A.; Hamavid, H.; Moradi-Lakeh, M.; Macintyre, M. F.; Allen, C.; Hansen, G.; Woodbrook, R.; *et al*. The Global Burden of Cancer 2013. *JAMA Oncol., 1(4)*:505-27, 2015.

Greco, A.; Rizzo, M.; De Virgilio, A.; Gallo, A.; Fusconi, M.; Pagliuca, G.; Martellucci, S.; Turchetta, R. & Vincentiis, M. Cancer stem cells in laryngeal cancer: what we know. *Eur. Arch. Otorhinolaryngol., 273(11)*:3487-95, 2016.

Grosse-Gehling, P.; Fargeas, C. A.; Dittfeld, C.; Garbe, Y.; Alison, M. R.; Corbeil, D. & Kunz-Schughart, L. A. CD133 as a biomarker for putative cancer stem cells in solid tumors: Limitations, problems and challenges. *J. Pathol., 229(3)*:355-78, 2013.

Huveneers, S.; Truong, H. & Danen, H. J. Integrins: signaling, disease, and therapy. *Int. J. Radiat. Biol., 83(11-12)*:743-51, 2007.

Katsamakas, S.; Chatzisideri, T.; Thysiadis, S. & Sarli, V. RGD-mediated delivery of small-molecule drugs. *Future Med. Chem., 9(6)*:579-604, 2017.

Kim, E. S.; Serur, A.; Huang, J.; Manley, C. A.; McCrudden, K. W.; Frischer, J. S.; Soffer, S. Z.; Ring, L.; New, T.; Zabski, S.; *et al*. Potent VEGF blockade causes regression of coopted vessels in a model of neuroblastoma. *Proc. Natl. Acad. Sci. U. S. A., 99(17)*:11399-404, 2002.

Li, F.; Liu, Y.; Kan, X.; Li, Y.; Liu, M. & Lu, J. G. Elevated expression of integrin alphav and beta5 subunit in laryngeal squamous-cell carcinoma associated with lymphatic metastasis and angiogenesis. *Pathol. Res. Pract., 209(2)*:105-9, 2013.

Li, Y. & Zhang, T. Targeting cancer stem cells by curcumin and clinical applications. *Cancer Lett., 346(2)*:197-205, 2014.

Lu, J. G.; Li, Y.; Li, L. & Kan, X. Overexpression of osteopontin and integrin alphav in laryngeal and hypopharyngeal carcinomas associated with differentiation and metastasis. *J. Cancer Res. Clin. Oncol., 137(11)*:1613-8, 2011.

Maccalli, C.; Volonte, A.; Cimminiello, C. & Parmiani, G. Immunology of cancer stem cells in solid tumours. A review. *Eur. J. Cancer, 50(3)*:649-55, 2014.

Miraglia, S.; Godfrey, W.; Yin, A. H.; Atkins, K.; Warnke, R.; Holden, J. T.; Bray, R. A.; Waller, E. K. & Buck, D. W. A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood, 90(12)*:5013-21, 1997.

Prince, M. E.; Sivanandan, R.; Kaczorowski, A.; Wolf, G. T.; Kaplan, M. J.; Dalerba, P.; Weissman, I. L.; Clarke, M. F. & Ailles, L. E. Identification of a subpopulation of cells with cancer stem cell properties in head and neck squamous cell carcinoma. *Proc. Natl. Acad. Sci. U. S. A., 104(3)*:973-8, 2007.

Pugh, C. W. & Ratcliffe, P. J. Regulation of angiogenesis by hypoxia: role of the HIF system. *Nat. Med., 9(6)*:677-84, 2003.

Rafii, S.; Lyden, D.; Benezra, R.; Hattori, K. & Heissig, B. Vascular and haematopoietic stem cells: novel targets for anti-angiogenesis therapy? *Nat. Rev. Cancer, 2(11)*:826-35, 2002.

Semenza, G. L. Evaluation of HIF-1 inhibitors as anticancer agents. *Drug Discov. Today, 12(19-20)*:853-9, 2007.

Shin, Y. C.; Lee, J. H.; Kim, M. J.; Park, J. H.; Kim, S. E.; Kim, J. S.; Oh, J. W. & Han, D. W. Biomimetic hybrid nanofiber sheets composed of RGD peptide-decorated PLGA as cell-adhesive substrates. *J. Funct. Biomater., 6(2)*:367-78, 2015.

Sun, C. C.; Qu, X. J. & Gao, Z. H. Integrins: players in cancer progression and targets in cancer therapy. *Anticancer Drugs, 25(10)*:1107-21, 2014.

Wang, J.; Wu, Y.; Gao, W.; Li, F.; Bo, Y.; Zhu, M.; Fu, R.; Liu, Q.; Wen, S. & Wang, B. Identification and characterization of CD133+CD44+ cancer stem cells from human laryngeal squamous cell carcinoma cell lines. *J. Cancer, 8(3)*:497-506, 2017.

Wei, X. D.; Zhou, L.; Cheng, L.; Tian, J.; Jiang, J. J. & Maccallum, J. *In vivo* investigation of CD133 as a putative marker of cancer stem cells in Hep-2 cell line. *Head Neck, 31(1)*:94-101, 2009.

Xu, Q.; Liu, Y.; Su, S.; Li, W.; Chen, C. & Wu, Y. Anti-tumor activity of paclitaxel through dual-targeting carrier of cyclic RGD and transferrin conjugated hyperbranched copolymer nanoparticles. *Biomaterials, 33(5)*:1627-39, 2012.

Yu, H.; Kortylewski, M. & Pardoll, D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. *Nat. Rev. Immunol., 7(1)*:41-51, 2007.

Yu, Z.; Pestell, T. G.; Lisanti, M. P. & Pestell, R. G. Cancer stem cells. *Int. J. Biochem. Cell Biol., 44(12)*:2144-51, 2012.

Zhang, Z.; Neiva, K. G.; Lingen, M. W.; Ellis, L. M. & Nor, J. E. VEGF-dependent tumor angiogenesis requires inverse and reciprocal regulation of VEGFR1 and VEGFR2. *Cell Death Differ., 17(3)*:499-512, 2010.

Zhou, L.; Wei, X.; Cheng, L.; Tian, J. & Jiang, J. J. CD133, CD133, one of the markers of cancer stem cells in Hep-2 cell line. *Laryngoscope, 117(3)*:455-60, 2007.

Zhou, Z.; Wang, M.; Li, J.; Xiao, M.; Chin, Y. E.; Cheng, J.; Yeh, E. T.; Yang, J. & Yi, J. SUMOylation and SENP3 regulate STAT3 activation in head and neck cancer. *Oncogene, 35(45)*:5826-38, 2016.

Corresponding author:
Xudong Wei, PhD
Department of Otolaryngology-Head and Neck Surgery
Gansu Provincial Hospital
No.160 Donggang West Rd
Lanzhou 730000
Gansu Province - CHINA

E-mail: weixd93@lzu.edu.cn