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Mechanisms of OGT2115 inhibition of invasion and migration in KB oral cancer cells

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Abstract. – OBJECTIVE: The purpose of this study was to investigate the effect of the heparanase inhibitor OGT2115 on the tumorigenic properties of KB oral cancer cells.

MATERIALS AND METHODS: We treated KB cells with different concentrations of OGT2115. Then proliferation, invasion, and migration were detected using different assays. Cell cycle was explored using flow cytometry.

RESULTS: We found that the treatment inhibited proliferation, invasion, and migration in a dose-dependent manner. OGT2115 also increased the apoptosis of KB cells and blocked cell cycle in S-phase.

CONCLUSIONS: Our results support the use of the heparanase inhibitor OGT2115 to i[illegible]it several tumorigenic activities of KB oral cells.

Key Words:
Oral cancer, Heparanase inhibitor, Apoptosis, cells.

Introduction

Oral cancer is [illegible]of the mos[illegible]mon malignant tumors an[illegible]rbidity rank[illegible]th place among all es[illegible]al [illegible]tumors. The main treatment for [illegible]ral cancer [illegible]very combined with adjuvan[illegible]emotherapy[1][illegible]hough numerous treatm[illegible]options are availab[illegible]low, the survival and [illegible]gnosis [illegible]oral cancer patients are low, w[illegible]five-[illegible]survival rate of only 50%[4,5]. It is well [illegible]at the i[illegible]ion and migration abilities of m[illegible]ant t[illegible]rs are closely related to [illegible]osis. [illegible], it is relevant to explore not o[illegible]he mec[illegible]sm of tumor cell proliferation a[illegible]ptosis but also migration and invasion for [illegible]g the pathogenesis of oral cancer [illegible]nd develop targeted therapies. A previous study[6] [illegible]ported that heparanase (HPA) inhibitors like [illegible]2115 play an important role in regulating tum[illegible]neovascularization and the process of tumor cells moving through the extracellular matrix (ECM). Here, we [illegible]se[illegible]ferent concentrations of OGT2115 to [illegible]mi[illegible]ologic[illegible]pact on KB oral can[illegible]ells.

Materials and Methods

Oral Cancer KB Cell Culture

KB cells were bought from Shanghai Cell Bank. [illegible]M medium ([illegible]o, Grand Island, NY, USA), [illegible]in (Gibco, G[illegible]l Island, NY, USA), mylicon ([illegible] Grand Is[illegible], NY, USA), and fetal bovine seru[illegible] Season, Hangzhou, China). KB cells cu[illegible]MEM medium, 10% fetal bovine serum (FBS), 2 g/L NaHCO3, 1 in 5 IU/L penicillin, [illegible]00 mg/L streptomycin (all from Bio-sharp, [illegible]ina). The culture was continued for 48 h at 37°C saturation humidity, and 5% CO_2.

MTT Test

KB cells in the logarithm growth period were transferred to 96-well plate (100 l, 8,000 cells), cultured in 5% CO_2, 37°C, and saturated humidity environment to help with cell attachment. After 24 h, culture solution was changed to a 10% newborn calf serum culture solution, 0.8, 1.6, 3.2, 6.4 mol/L OGT2115 (Tocris Bioscience, Ellisville, MO, USA) and 10 µl (5 g/L) MTT (Sigma-Aldrich, St. Louis, MO, USA) were added to each well for the last 4 h. KB cells were cultured for 24, 48, and 72 h; the supernatant was removed, and 150 µl dimethyl sulfoxide (Biosharp, Hefei, China) was added in each well; then, the cells were incubated at 37°C for 30 min. An automatic quantitative graphics microplate reader (Bio-Rad, Hercules, CA, USA) was used to make readings at 570 mm.

Cell Apoptosis Flows Cytometry FCM-test

High activity cell suspension was prepared. 10% fetal calf serum (FCS) Roswell Park Memorial Institute (RPMI)-1640 was used to adjusting the H1299 cell concentration to 5× 10^6-1×10^7/ml.

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40 µl centrifuged cell suspension was stored in a specific McAb (5-50 µl) glass tube. The rabbit serum was inactivated at 4°C for 30 min and washed twice with 3 ml for each time; then, it was centrifuged at 1,000 rpm × 5 min. 50 µl rabbit-anti-rat fluorescent marked material was treated at 4°C for 30 min, fixed after washing twice. The slides were prepared and observed with a fluorescent microscope (IX70, Olympus, Tokyo, Japan).

Cell Cycle Test

The cells were inoculated with 1×10^5/ well into 6-well plate; trypsin-EDTA is used for cell dissociation after culturing for 24 h. The cells were centrifuged at 400 x g and were resuspended in 300 µl-4°C pre-cooled phosphate buffered saline (PBS); the cells were mixed and 700 µl -20°C pre-cooled ethyl-alcohol (Et-OH) was added drop by drop to fix the cells, and were stored overnight at -20°C. The cells were centrifugated and precipitated; then, were followed by washing with PBS; the supernatant was removed and resuspended in a 500 µl Propidium Iodide PI/RNase Staining Buffer Solution; finally stained in a darkroom for 15 min. FACS Flow Cytometry (FCM) (BD Bioscience troit, MI, USA) was used to detect the cell c The data was analyzed by using Modfit LT.

Cell Migration Test

The basement membra aterial s artificially rebuilt with Matr Sigma St. Louis, MO, USA). Typ DT Shanghai, China) w sed dissociation followed by cen gation of Then, the cells were res d in serum- edium; the cell con atio adjusted t 2×10^5/ml; 600-800 u serum-supp edium was incubated in 24 l plates and st a chamber with 100-1 l cell suspension. continued incuba them f 2-24 hours, we removed the c om t hambe with tweezer, we dryed out amber id, rub out the upper amb by u cotton swab; chamber is d in or 15 min, then dry in air, pl the cha in 0.1% crystal violet staining s for staining 15 min, after washing with photograph and count the amount cells to evaluate the migration situations.

swell Test

answell Cabin (24-pore, 0.8 µm) (Corning, New York, NY, USA) microfiltration culture chamber and double-chamber co-culture system were used. Cell cultivation density was 1 cell. The experiment procedures followed th dardized specifications provided by th

Statistical Analysis

SPSS13.0 package (IBM, Armon USA) was used for statistical analy We us n ± standard error (± SD) to ent the data analysis was performed sing r titive m surement and analysi varian ndepende sample *t*-test was use th ta eva on. $p < 0.05$ was re d as ndard sig- nificant differe Pearson d chosen for correlati sis.

Re

2115 Effect on KB Cell Viability

o test the ef of heparanase inhibition on rowth of K ral cancer cells, we treated th ls with O 115. We used four different con OGT2115 (0.4, 0.8, 1.6, 3.2, and 6.4) and determined growth for each ncentration 24, 48, and 72 h later (Figure 1). ves shown that all concentrations of 5 were effective, particularly at 72 h. Treatments above 1.6 µg/L OGT2115 showed clear differences at 24 h, with larger effects at 48 and 72 h (Figure 1). Overall, all the curves demonstrated statistically significant differences ($p < 0.05$).

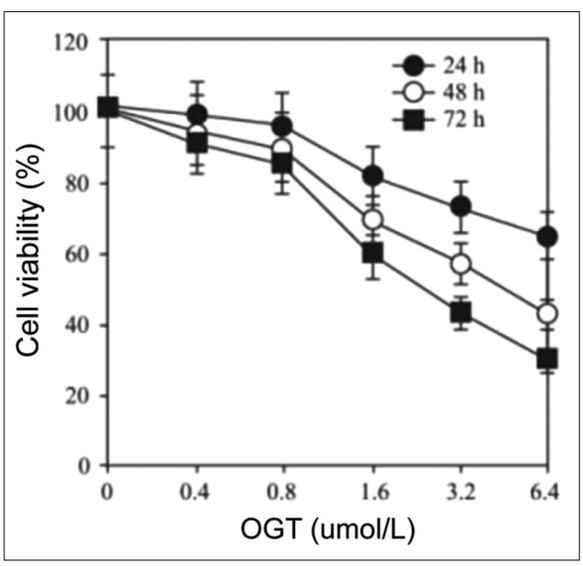

Figure 1. Effect of OGT2115 on KB cell viability.

OGT2115 Inhibits KB Cell Invasion and Migration

Next, we used the Transwell method to analyze the effect of OGT2115 on the ability of KB cells to migrate. For this experiments, we used less than half of the IC50 concentration of OGT2115, which has little effect on cell viability. After treating KB cells with OGT2115 at 0.4, 1.6, and 3.2 μmol/L for 24h and 48 h, we fixed and stained the slides to detect the invading and migrating cells. We photographed and counted the cells at 200X. OGT2115 at 0.4 μmol/L had no effect on invasion and migration (Figure 2 A-B). However, OGT2115 at 1.6 and 3.2 μmol/L reduced the amount of invading and migrating cells, with the 3.2 μmol/L treatment showing the most robust effects (Figure 2 A-B). OGT2115 treatments above 1.6 μmol/L [illegible] strated the effective inhibition of i[illegible]sion and migration.

Cell Apoptosis Test

We next examined the [illegible]t of OGT2[illegible] cell death of KB cells. W[illegible]eated [illegible]B cells [illegible] OGT2115 at 0, 0.4, [illegible]nd 3.2 [illegible]ol/L for 24 and 48 h and determi[illegible]o[illegible]s by fl[illegible]cytometry. The c[illegible]rol [illegible]ment[illegible]hout OGT2115 sho[illegible]no apopt[illegible]ell[illegible]gure 3

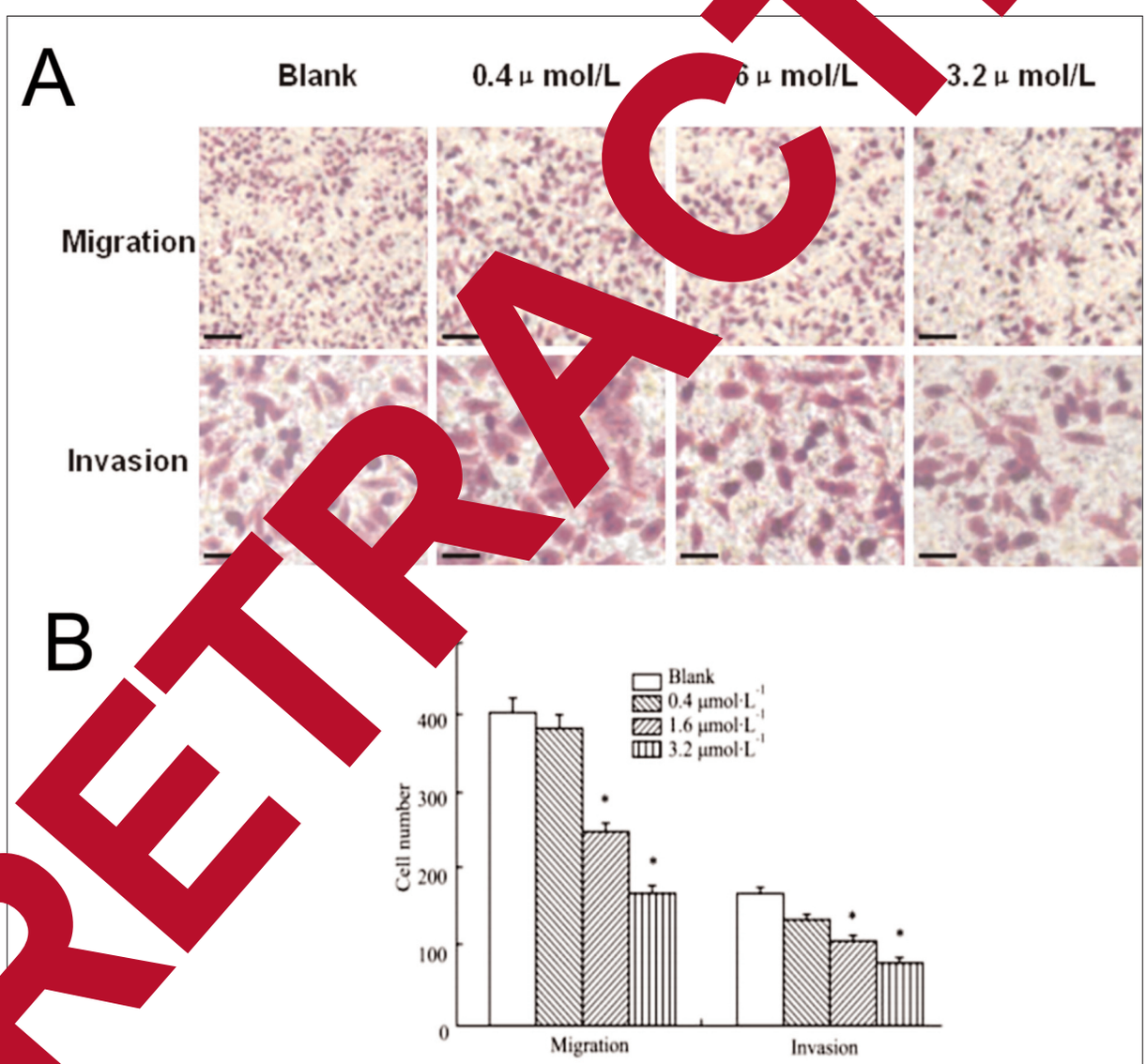

Figure 2. ***A,*** Invasion and migration of KB cells treated with OGT2115 for 24 and 48 h. ***B,*** Quantification of KB cells at 200× and 400× under the light microscopes.

A-B). The 0.4 μmol/L OGT2115 induced apoptosis in 10% of the cell population (Figure 3 A-B), indicating that even low concentrations of OGT2115 alter the properties of KB cells. The 1.6 and 3.2 μmol/L OGT2115 treatments induced further apoptosis, demonstrating a dose-sensitivity to OGT2115 concentration (Figure 3 A-B).

OGT2115 Stabilizes KB Cells in S-phase

Lastly, we examined the effect of OGT2115 on cell cycle using the same conditions as in the apoptosis experiment. The 1.6 and 3.2 μmol/L OGT2115 treatments stabilized KB cells at S-phase and reduced the percentage of cells in G1 (Figure 4 A-B).

Discussion

Oral cancer is one of the most common tumors of the digestive system. These cancers affect the quality of life, appearance, and psychology of patients[7,8]. The invasion and migration of cancer cells is one of the important biological events and also the main cause of early death and low survival of patients. Invasion and migration
cer cells is a complicated, multifac
continuous biological process, but t
mechanisms regulating these ever
completely understood[9-11]. The invasio
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three main biological pro
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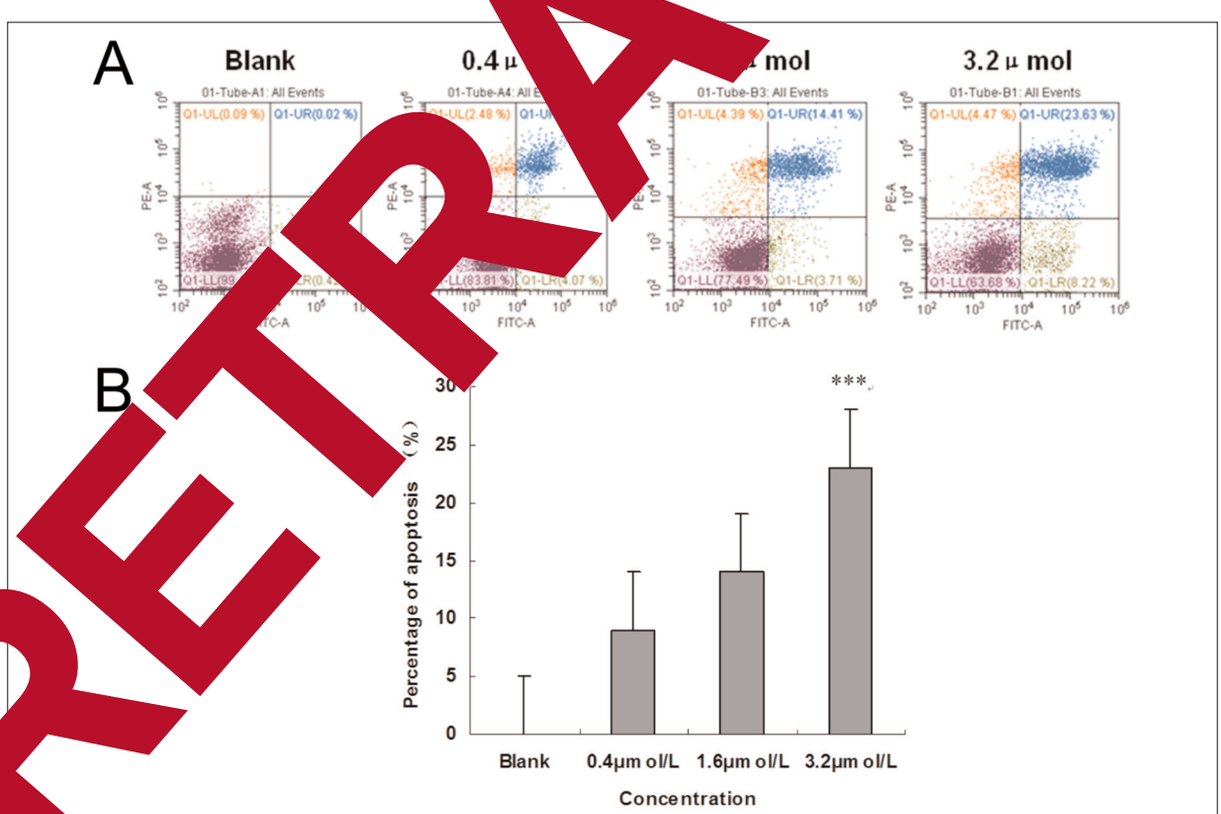

Figure 3. ***A,*** Percentage of KB cell apoptosis treated by OGT2115 for 48 h detected by FCM. ***B,*** Cell number of PE-A+FITC-A+.

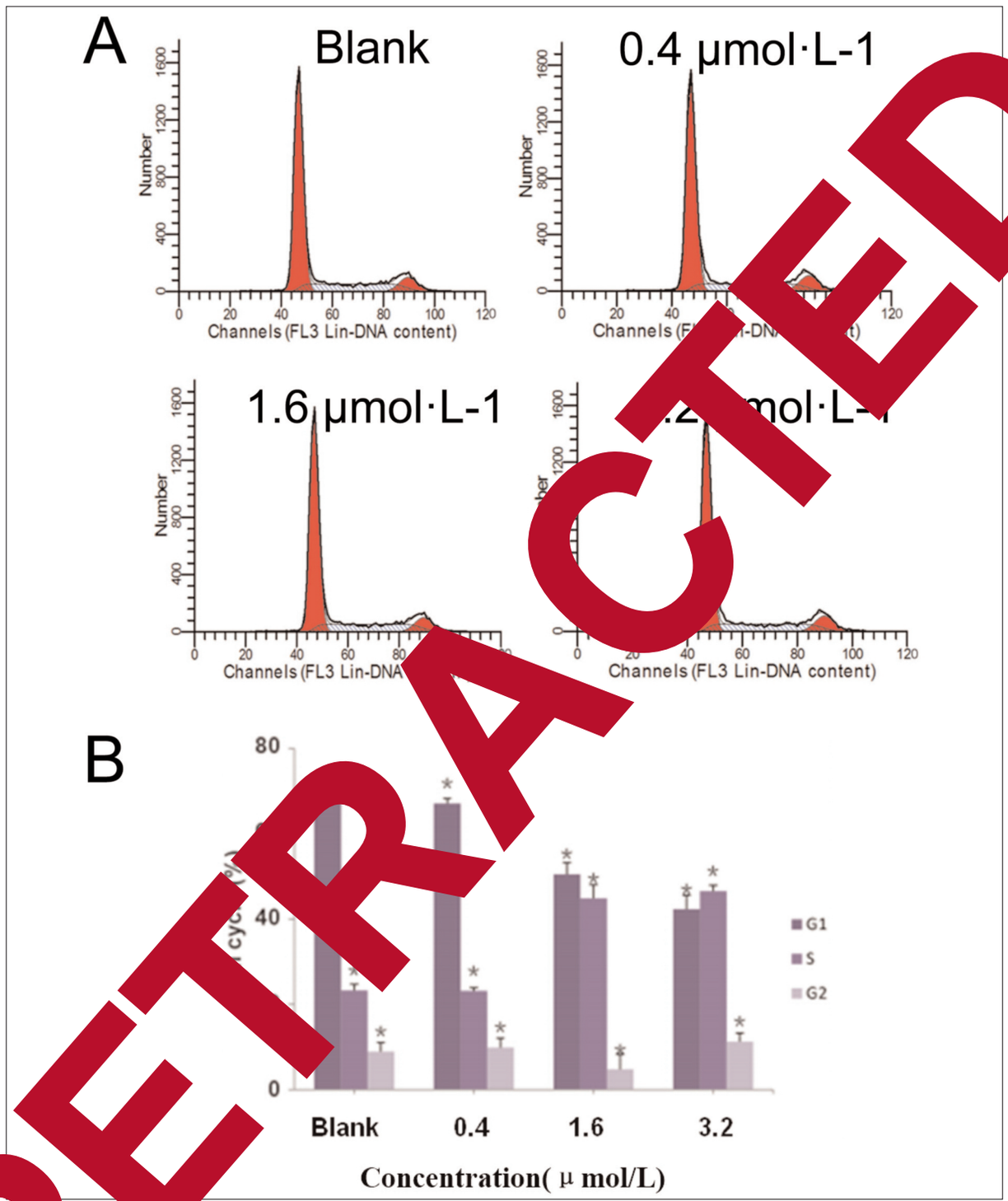

Fig. 4. ***A,-B,*** K cell cycle detected by FCM after the treatment of OGT2115 for 48 hours.

e generation of HPA. This is due to the ability HPA to bind with side chain locus specifically HSPG, which can inhibit the tumor neovascularization in rat tumor tissues, can be generated in the following chain scission[17-19]. Our results show that KB cell treated with OGT2115 for 48 hours presented lower cell viability, invasion, and migration in a dose-dependent manner. Moreover, OGT2115 increased apoptosis and blocked KB cells in S-phase of the cell cycle[20].

Conclusions

OGT2115 demonstrated anti-cancer activity by inducing cancer cells apoptosis and inhibiting invasion. Page MJ found that oral nucleoside analogs like OGT2115 can inhibit the invasive growth of colon cancer cells and liver cells.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009; 45: 309-316.
2) Ram H, Sarkar J, Kumar H, Konwar R, Bhatt ML, Mohammad S. Oral cancer: risk factors and molecular pathogenesis. J Maxillofac Oral Surg 2011; 10: 132-137.
3) Meng J, Wang X, Zhuang QW, Gu QP, Zhang J, Li ZP. Clinical effectiveness of 125I-seed implantation in combination with nimotuzumab ther[illegible] for the advanced oral carcinoma: preliminar[illegible] Eur Rev Med Pharmacol Sci 2014; 18[illegible] 3310.
4) Chaturvedi AK, Anderson WF, Lortet-Tieulent J[illegible] rado MP, Ferlay J, Franceschi S, Rosenberg PS, [illegible] F, Gillison ML. Worldwide t[illegible] incidenc[illegible] rates for oral cavity and orc[illegible] cancers. [illegible] Clin Oncol 2013; 31: 4550[illegible]9.
5) He C, Wei Y, Sun K, L[illegible] Dong X, [illegible] Z, Liu Y, Kinch LN, Khan S, Sinha[illegible] ier R[illegible] ao G, Eskelinen El[illegible] her[illegible] ler JL, Le[illegible] ne B. Beclin 2 funct[illegible] in autop[illegible] degradation of G protein-co[illegible] receptors, [illegible] metabolism. Cell 2013; 1[illegible]-1099.
6) Li Y, Liu L[illegible] ang[illegible] LJ, Zhang X[illegible], Jiang CC, Jiang ZW. Suppress[illegible] endoplasmic reticulum stres[illegible] duced invasio[illegible] migration of breast ca[illegible] cells through the [illegible] regulation of he-[illegible]ase. Int J Mol Med 2013; 31: 1234-1242.
7) [illegible]aturved[illegible] Anderson WF, Lortet-Tieulent J, Cu-[illegible] MP[illegible]lay J, Fr[illegible]eschi S, Rosenberg PS, Bray [illegible] ML. Wo[illegible]e trends in incidence rates for [illegible]vity a[illegible]opharyngeal cancers. J Clin Oncol[illegible]2[illegible]50-4559.
8) [illegible]un HB, [illegible] HY, Yan X. Survivin silencing en-[illegible]ances radiosensitivity in oral squamous cell car-[illegible]. Eur Rev Med Pharmacol Sci 2014; 18: [illegible]2686.
9) Chiang CH, Huang CC, Chan WL, Huang PH, Chen TJ, Chung CM, Lin SJ, Chen JW, Leu HB. Oral alendronate use and risk of cancer in [illegible] menopausal women with osteoporosis[illegible] wide study. J Bone Miner Res 201[illegible]: 1951-1958.
10) Raman K, Kuberan B. Chemical [illegible]biology of heparan sulfate proteoglycans. C[illegible]m Biol 2010; 4: 20-31.
11) Hermano E, Lerner I, Elkin[illegible] heparanase [illegible] in chronic inflammator[illegible]vel dise[illegible]se and [illegible] cancer. Cell Mol Life [illegible]012; 69[illegible]1-2513.
12) Lee JH, Kang JG, S[illegible] Jeo[illegible], Oh S, [illegible]m YS, Ko JH. N-Acet[illegible]luc[illegible]nsferas[illegible] triggers overexp[illegible]on of M[illegible]MP an[illegible]morces the invasiv[illegible]tastatic pot[illegible]of[illegible]cer cells. Biochem[illegible] Res Comm[illegible] 431: 658-663.
13) Zhang [illegible], Yang [illegible]ang Q, Yang ZJ, Chen H, Wang SM, Pan ZM, [illegible]BJ, Li QQ, Li L. Clinical [illegible]combined dete[illegible]of serum matrix metalloproteinase-9, heparan[illegible]se, and cathepsin for determining ovarian cancer invasion and metastasis. Anticance[illegible]s 2011; 31: 3423-3428.
[illegible]hen Y, Li X, W[illegible], He C, Guo L, Zhang S, Xiao Y, [illegible]o W, Tan [illegible]utophagy-related proteins LC3 [illegible]eclin[illegible]pact the efficacy of chemoradiation[illegible]ngeal squamous cell carcinoma. Pathol Res Pract 2013; 209: 562-567.
Zhang W, Yang HC, Wang Q, Yang ZJ, Chen H, [illegible]SM, Pan ZM, Tang BJ, Li QQ, Li L. Clinical [illegible]e of combined detection of serum matrix metalloproteinase-9, heparanase, and cathepsin for determining ovarian cancer invasion and metastasis. Anticancer Res 2011; 31: 3423-3428.
16) Berger CE, Qian Y, Liu G, Chen H, Chen X. P53, a target of estrogen receptor (ER) alpha, modulates DNA damage-induced growth suppression in ER-positive breast cancer cells. J Biol Chem 2012; 287: 30117-30127.
17) Ishikawa S, Sugimoto M, Kitabatake K, Sugano A, Nakamura M, Kaneko M, Ota S, Hiwatari K, Enomoto A, Soga T, Tomita M, Iino M. Identification of salivary metabolomic biomarkers for oral cancer screening. Sci Rep 2016; 6: 31520.
18) Ma LY, Liu H, Jiang ZW. The research progress of heparin and HS corresponding anti-cancer drugs. Chin Pharmacol Bull 2012; 1193-1196.
19) Sanderson RD, Iozzo RV. Targeting heparanase for cancer therapy at the tumor-matrix interface. Matrix Biol 2012; 31: 283-284.
20) Rohlff C, Watson SA, Morris TM, Skelton L, Jackman AL, Page MJ. A novel, orally administered nucleoside analogue, OGT 719, inhibits the liver invasive growth of a human colorectal tumor, C170HM2. Cancer Res 1999; 59: 1268-1272.