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HMTH1 induces the metastasis and recurrence of the parotid adenoma by repairing DNA damage

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Abstract. – OBJECTIVE: To investigate the effect of hMTH1 (human mutT homologue 1) on inducing the metastasis and recurrence of parotid adenoma, which may provide a new therapeutic direction for the prevention and treatment of parotid adenoma.

PATIENTS AND METHODS: 30 cases of paraffin-embedded specimens of parotid adenoid cystic carcinoma (ACC) tissues and fresh parotid glands surgically resected in our hospital were collected as experimental group. 30 cases of surgically resected pleomorphic adenoma (PA) in the same period were selected as another experimental group. Meanwhile, 30 cases of normal parotid gland tissues (N) were collected as control group. The mRNA and protein expressions of hMTH1 in parotid gland tissues of patients with parotid adenoma before and after surgery were detected by Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) and Western blotting, respectively. HMTH1 expression levels in parotid gland tissues were also detected by immunohistochemistry. Proliferation, apoptosis and DNA damage of ACC-M cells treated with S-Crizotinib were detected by cell counting kit-8 (CCK-8) assay, flow cytometry and single cell gel electrophoresis, respectively.

RESULTS: Both mRNA and protein expressions of hMTH1 in experimental group were significantly higher than those of control group. Moreover, a higher expression of hMTH1 was observed in ACC than that of PA, indicating that hMTH1 expression was positively correlated with the malignant degree of parotid adenoma. Furthermore, postoperative hMTH1 expression levels in patients with parotid adenoma were significantly lower than those before treatment, which were remarkably increased in recurrent patients. *In vitro* experiments demonstrated that S-Crizotinib, the hMTH1 inhibitor, could inhibit proliferation and induce apoptosis and DNA damage of ACC-M cells.

CONCLUSIONS: HMTH1 was upregulated in patients with parotid adenoma and recurrent patients after surgery. Meanwhile, S-Crizotinib induced DNA damage in ACC-M cells, indicating that hMTH1 induced the metastasis and recurrence of parotid adenoma by repairing DNA damage, providing a new strategy for the prevention and treatment of parotid adenoma.

Key Words:
HMTH1, Parotid adenoma, DNA damage.

Introduction

Since DNA damage can lead to changes in genetic information, there is widespread concern about the relationship between DNA damage along with DNA repair mechanism and related diseases[1]. Base excision repair (BER) is one of the most important repair methods of DNA damage[2]. So far, there are more than 100 enzymes involved in the repair of DNA damage, of which, 8-oxoguanine nucleotide triphosphatase is one of the most studied enzymes[3].

HMTH1 (human mutT homologue 1), a member of the nudix hydrolase family, could prevent DNA damage by inhibiting the insertion of various oxidized nucleotides into DNAs or RNAs. Studies have shown that hMTH1 is upregulated in a variety of tumor tissues. Borrego et al[4] demonstrated that mRNA expression of hMTH1 in gastric adenocarcinoma is remarkably increased. In addition, mRNA expression of hMTH1 is also significantly increased in pancreatic cancer and non-small cell lung cancer[5,6]. Another investigation[7] elucidated that HMTH1 activity is significantly enhanced in lung cancer. Previous studies have consistently suggested that hMTH1 may exert an essential role in tumor cell growth. Gad et al[8] demonstrated that hMTH1 could lead to DNA damage and reduce survival ability of cancer

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cells, indicating that hMTH1 was necessary for the integration of deoxy-ribonucleotide triphosphates (dNTPs) into DNA and the DNA oxidative damage resulting from it. However, overexpression or knockdown of hMTH1 in primary VH10 cells did not affect cell survival, suggesting that hMTH1 affects cancer cells rather than normal cells.

Currently, hMTH1 inhibitors have been reported[9,10] to be able to integrate oxidized dNTPs into DNAs in cancer cells, thus leading to DNA damage and cytotoxicity, providing a new strategy for the treatment of cancer.

A large number of researches have shown that hMTH1 is closely related to the occurrence and development of tumor. However, there is little research on hMTH1 in parotid adenoma. This study explored the effect of hMTH1 on the development and progression of parotid adenoma by detecting hMTH1 expression in patients with parotid adenoma. Meanwhile, the effect of S-crizotinib on biological functions of parotid gland carcinoma cell line was also investigated, which may provide new basis for treating the aggressiveness parotid adenoma.

Patients and Methods

Patients

A total of 30 cases of paraffin-embedded specimens of parotid adenoid cystic carcinoma (ACC) tissues and fresh parotid glands surgically resected in our hospital from July 2005 to February 2016 were collected as experimental group. 30 cases of surgically resected pleomorphic adenoma (PA) in the same period were also selected as experimental group. Meanwhile, 30 cases of normal parotid gland tissues were collected as control group. Enrolled patients were 9 to 80 years old, with an average age of 41 years. All specimens were pathologically diagnosed. Histopathology and grading of parotid adenoma were based on WHO-2005 classification criteria, and TNM staging was based on UICC-2002 standard. Normal parotid gland tissues were taken from patients who underwent cervical lymphadenectomy, which were then embedded with paraffin as control group. Approved by the Ethics Committee of The Second Affiliated Hospital of Xinjiang Medical University. Signed written informed consents were obtained from all participants before the study.

ACC-M Cell Culture

ACC-M cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 U/mL streptomycin. Cells were placed in a 5% CO_2 incubator at 37°C. ACC-M cells were digested with 0.25% trypsin and passaged with 0.02% EDTA (Ethylene Diamine Tetraacetic Acid) every 72 h and 96 h.

Pathological Examinations of Parotid Adenoma

Hematoxylin and eosin (HE) staining was performed in pleomorphic adenoma (PA), adenoid cystic carcinoma (ACC) and normal parotid tissue (N) for pathological grading. HMTH1 expression was detected by S-P immunohistochemical staining and brown vascular endothelial cell clusters were observed under a 200× light microscope. All tumor cells with hMTH1 positively expressed were brown and hMTH1 dyed brown was located in the cytoplasm. The proportion of positive stained cells was calculated in 10 randomly selected cases of high power field. Specifically, < 10% was defined as negative (-); 10-50% was considered as positive (+); and > 50% was regarded as strongly positive (++).

Western Blotting

Total protein was extracted using a cell lysate (RIPA) (Beyotime, Shanghai, China). Protein samples were separated in a sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and transferred to a polyvinylidene fluoride (PVDF) membrane (Roche, Basel, Switzerland), which was blocked with the blocking solution for 1 h. Primary antibody (anti-hMTH1, 1:1000) was used for incubation at room temperature for 2 h. After washed with Tris-buffered saline-tween (TBS-T), corresponding secondary antibody (horseradish peroxidase (HRP)-labeled IgG antibody, 1:10000) was used for incubation for 2 h at room temperature. Immunoreactive bands were visualized by enhanced chemiluminescence.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA in cells was extracted by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA) according to

the instructions. Each sample was repeated for 3 times. Primers used in this study were listed in Table I.

Flow Cytometry

Annexin V+/7-AAD- was added into the resuspended cell suspension according to the instructions of the apoptosis kit. All antibodies were purchased from eBioscience (San Diego, CA, USA). After incubation for 15 min in the dark, cells were centrifuged at 400 g for 5 min, followed by the apoptosis detection by flow cytometry.

Single Cell Gel Electrophoresis (SCGE)

Cells were transfected for 48 h and centrifuged at 700 g for 2 min. Cell density was adjusted to 1 × 10^5/mL with pre-cooled PBS (phosphate-buffered saline). 10^5 cells were mixed with 200 μL of agarose. 75 μL of mixture were blown out with a pipette on OxiSelect™ Comet Slide. Electrophoresis was performed and data were analyzed using a fluorescent microscope.

Statistical Analysis

Graphpad Prism (v6.0, La Jolla, CA, USA) was used for data analysis. Measurement data were presented as mean ± standard deviation. Comparison of measurement data was performed using *t*-test. Each experiment was repeated for 3 times. One-way ANOVA was performed to identify differences among multiple groups and Bonferroni was used to analyze the significance of each group. $p < 0.05$ was considered statistically significant.

Results

HMTH1 Eas Involved in the Pathological Formation of Parotid Adenoma

To investigate whether hMTH1 was involved in the pathological formation of parotid adenoma, mRNA (Figure 1A) and protein expressions of hMTH1 (Figure 1B) in parotid gland tissues from patients with parotid adenoma and normal controls were detected by qRT-PCR and Western blot, respectively. Higher mRNA and protein expressions of hMTH1 were observed in parotid gland tissues of patients with parotid adenoma than those of normal controls. Moreover, hMTH1 expression was positively correlated with the malignant degree of parotid adenoma, indicating that hMTH1 might participate in the pathological formation of parotid adenoma.

Meanwhile, we detected protein (Figure 1C) and mRNA (Figure 1D) expression levels of hMTH1 in N and ACC before and after treatment by Western blot and qPCR, respectively. Additionally, hMTH1 expressions in recurrent parotid gland tissues were also detected. Our data indicated that postoperative hMTH1 expressions in patients with parotid adenoma were remarkably lower than those before treatment, but they were increased again in recurrent patients. It was suggested that hMTH1 was involved in the pathological formation of parotid adenoma and positively correlated with malignant degree of parotid adenoma.

HMTH1 Participated in the Invasion and Metastasis of Parotid Adenoma

To further explore the effect of hMTH1 on the development and progression of parotid adenoma, patients with parotid adenoma were divided into different groups based on the pathological classification. Histological analysis showed that hMTH1 was upregulated in the parotid gland tissue of patients with parotid adenoma compared with that of normal controls (Figure 2A). Furthermore, higher hMTH1 expression was found in ACC with higher degree of malignancy (Figure 2B) in comparison with that in PA with lower degree of malignancy (Figure 2C), indicating that hMTH1 was not only involved in the pathological formation of parotid adenoma, but also closely associated with the invasiveness ability.

HMTH1 Promoted Proliferation of Parotid Adenoma Cells

After confirming that hMTH1 was capable of affecting the metastasis and invasion of pa-

Table I. RT-qPCR primer pairs.

Name	Forward	Reverse
GAPDH	CATCAAGAAGGTGGTGAAGCAG	CAAAGGTGGAGGAGTGGGTG
hMTH1	AGGTGGGCCAGATCGTGTTTG	TCGTCGGGCCACATGTCC

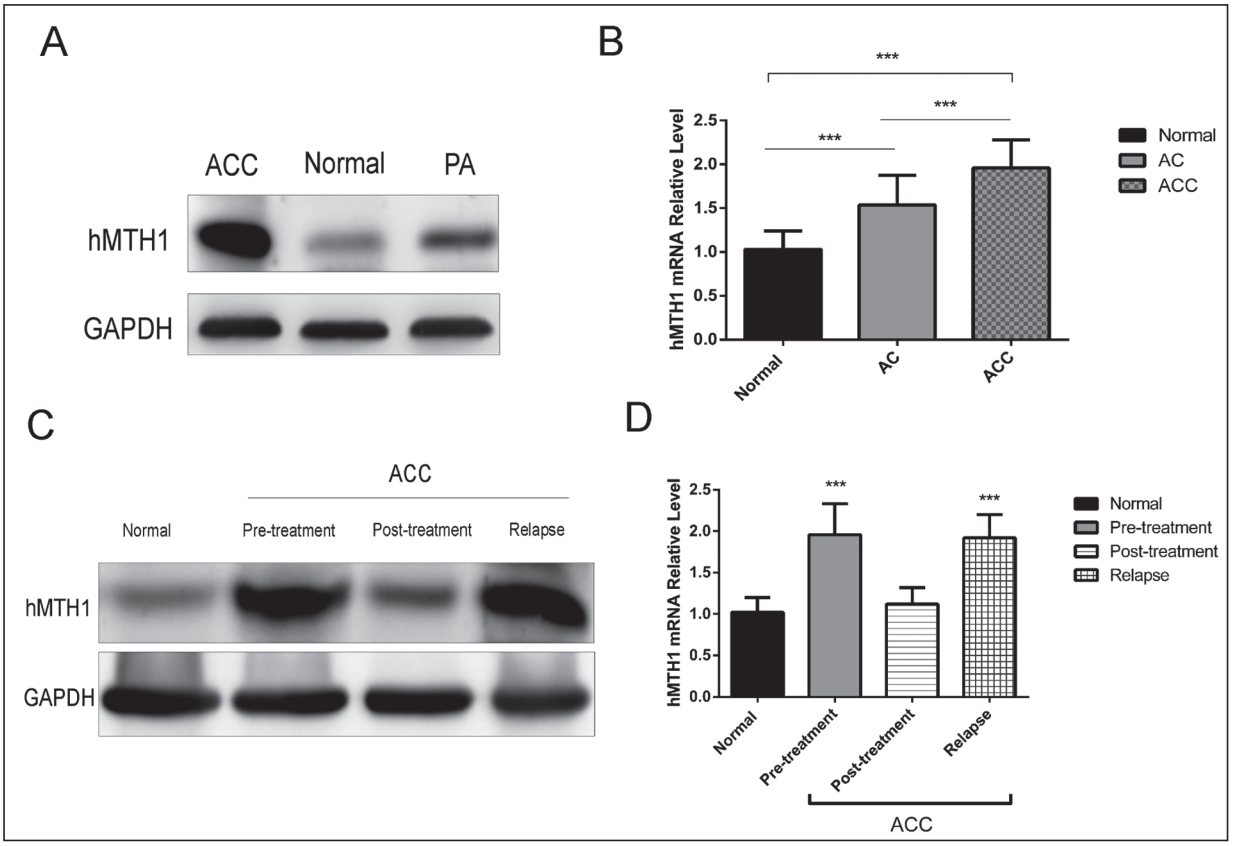

Figure 1. HMTH1 participates in the pathological formation of parotid adenoma. ***A,*** Protein expressions of hMTH1 in each group were detected by Western blot. ***B,*** The mRNA level of hMTH1 in N, ACC and PC were detected by qRT-PCR. ***C,*** Protein expressions of hMTH1 in N, ACC patients before and after surgery and recurrent ACC patients were detected by Western blot. ***D,*** The mRNA level of hMTH1 in N, ACC patients before and after surgery and recurrent ACC patients were detected by qRT-PCR. Each group had 20 samples (***$p < 0.001$).

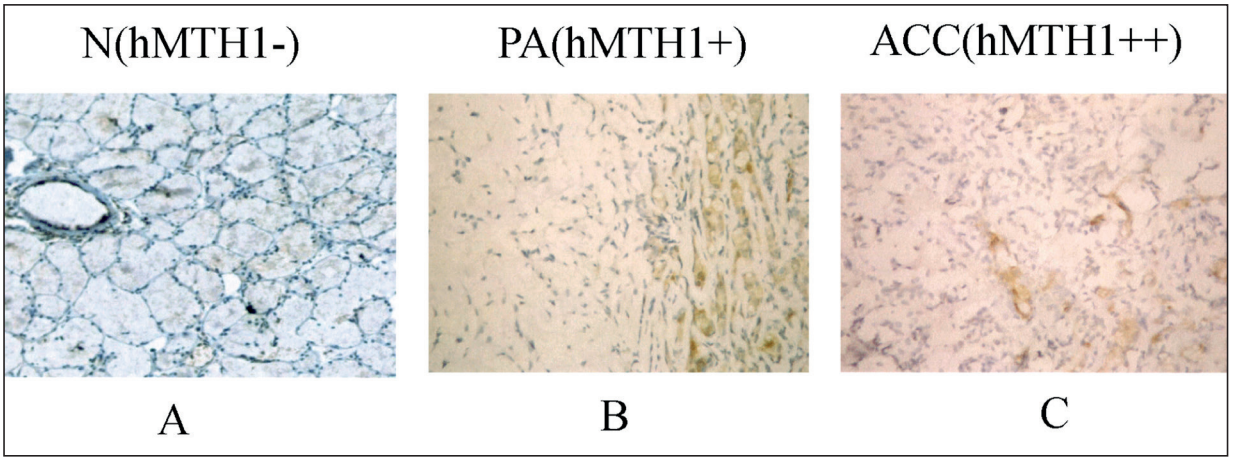

Figure 2. HMTH1 participated in invasion and metastasis of parotid adenoma. PA, ACC and N were selected for S-P immunohistochemistry. Negative hMTH1 was found in N (***A,***) and positive hMTH1 was found in PA (***B,***) and ACC (***C,***).

rotid adenoma, we further investigated whether hMTH1 may directly affect the proliferation of parotid adenoma cells.

ACC-M cells were first treated with different concentrations of S-Crizotinib (0.125, 0.25, 0.5, 1, 2, 4, 6 and 8 μmol/L) for 48 h, followed by the detection of cell apoptosis using CCK-8 assay (Figure 3A). Moreover, Pearson linear correlation analysis indicated that S-Crizotinib concentration was positively correlated with the rate of apoptosis ($r = 0.911$, $p < 0.01$). Our data suggested that the apoptotic rate of ACC-M cells treated with different concentrations of S-Crizotinib was remarkably increased compared with that of the control group ($p < 0.05$, Figure 3B). Taken together, our results showed that S-Crizotinib promoted apoptosis of ACC-M cells in a dose-dependent manner, indicating that hMTH1 directly participated in the proliferation of parotid adenoma cells.

HMTH1 Induced Metastasis and Recurrence of the Parotid Adenoma by Repairing DNA Damage

Studies[11] have shown that hMTH1 exerts a crucial role in the repair of DNA damage. Therefore, SCGE was performed after ACC-M cells were treated with S-Crizotinib for 48 h (Figure

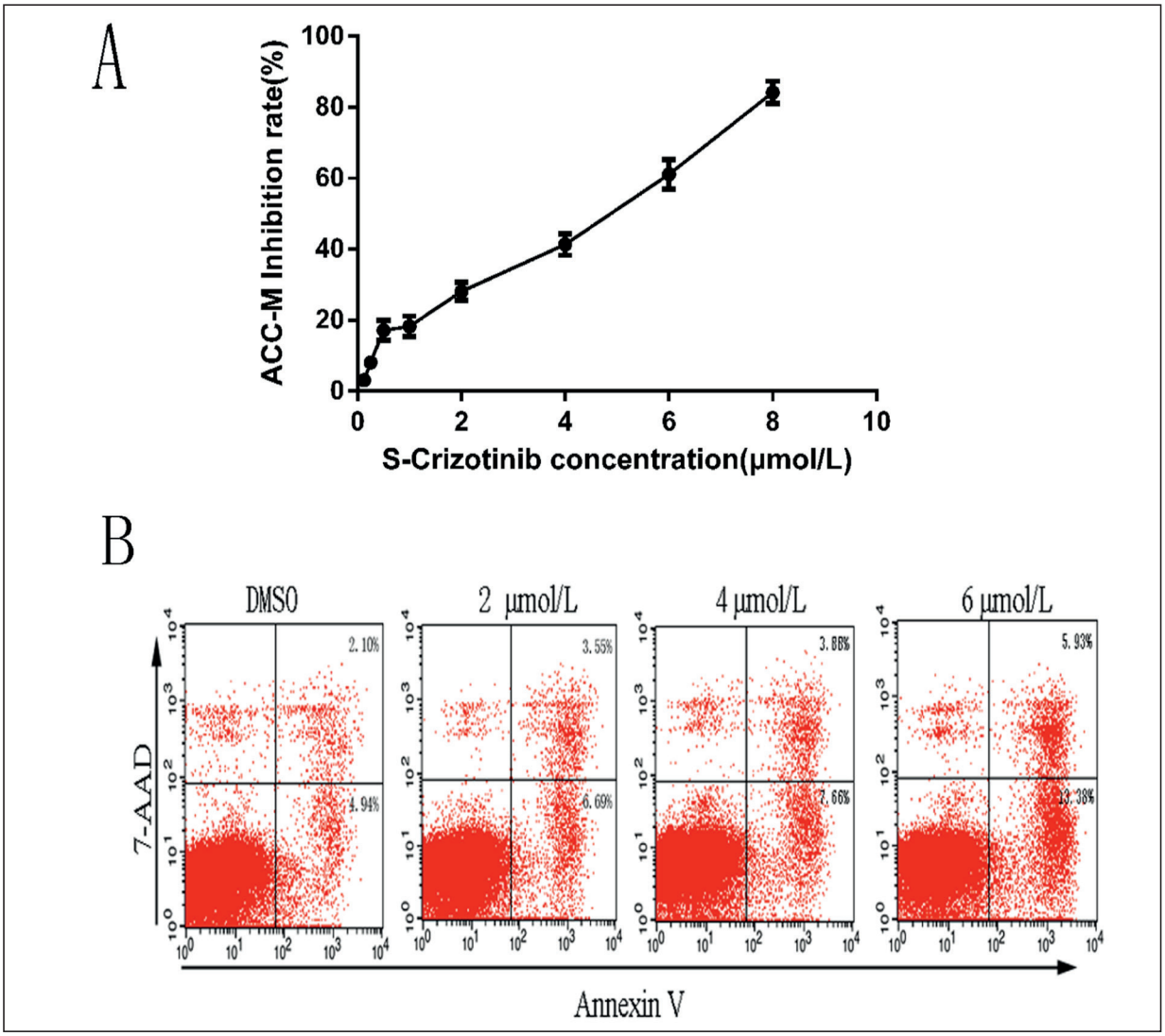

Figure 3. HMTH1 promoted proliferation of parotid adenoma cells. ***A,*** Apoptosis rate of ACC-M cells after treated with different concentrations of S-Crizotinib (0.125, 0.25, 0.5, 1, 2, 4, 6 and 8 μmol/L) for 48 h was detected by CCK-8 assay. ***B,*** Cell apoptosis of ACC-M cells after treated with different concentrations of S-Crizotinib (2, 4 and 6 μmol/L) for 48 h was detected by flow cytometry (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

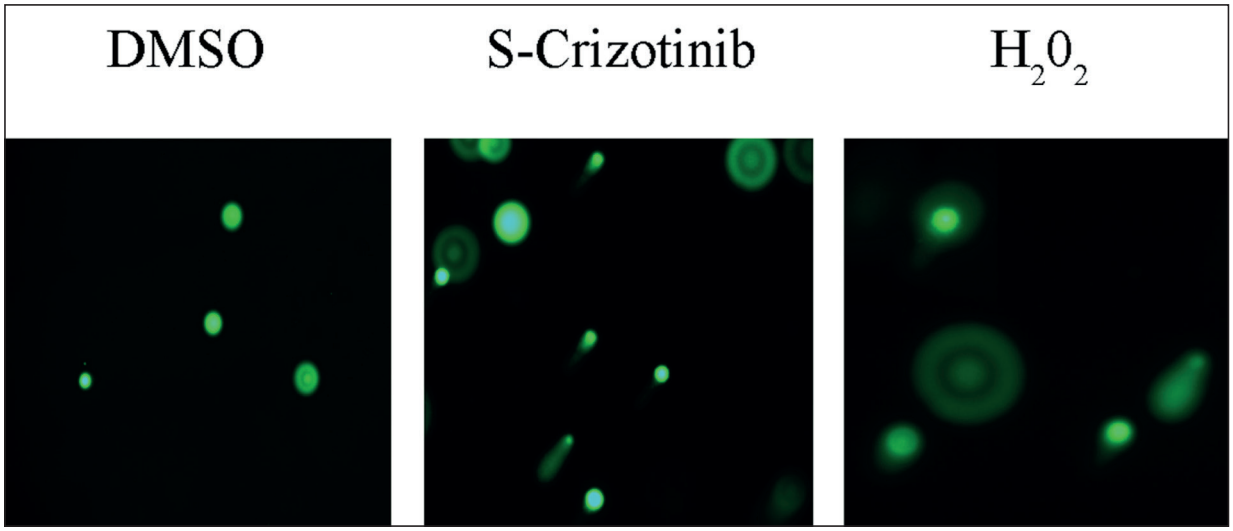

Figure 4. HMTH1 induced metastasis and recurrence of parotid adenoma by repairing DNA damage. SCGE was performed after ACC-M cells were treated with S-Crizotinib for 48 h, with H_2O_2 treatment as controls.

4). SCGE results showed that S-Crizotinib could lead to DNA damage of parotid adenoma cells, indirectly confirming that hMTH1 could induce the metastasis and recurrence of parotid adenoma by repairing DNA damage.

Discussion

HMTH1 is an important enzyme in the process of DNA repair, which can hydrolyze the oxidized deoxyribonucleotides (dNTPs) in nucleotide pools. For example, hMTH1 can convert 8-oxo-dGTP and 2-OH-dATP to 8-oxo-dGMP and 2-OH-dAMP, respectively, thus preventing DNA damage[11]. The mRNA levels of hMTH1 in tumor tissues, including brain cancer[12], breast cancer[13], lung cancer[14,15], gastric cancer[4] and kidney cancer[16], are higher than those in paracancerous tissues.

In this study, higher mRNA and protein levels of hMTH1 were observed in parotid adenoma than those of normal controls. Furthermore, hMTH1 expression was positively correlated with the malignancy of parotid adenoma. Additionally, postoperative hMTH1 expression was declined to the normal level, which was reversed to a high level again in recurrent patients. Our data indicated that hMTH1 was not only involved in the pathological formation of parotid adenoma, but also closely related to its invasive ability.

Gad et al[8] and Huber et al[10] found that hMTH1 was necessary in cancer cells but not in normal cells. Inhibition of hMTH1 caused DNA damage and cell death in cancer cells, suggesting that hMTH1 could be served as a target for cancer therapy. Our study showed that hMTH1 expression levels were significantly increased both in preoperative patients and recurrent patients with parotid adenoma. However, many chemotherapeutic drugs can induce reactive oxygen species in tumor cells[17-19], thereby causing death of tumor cells. Multiple studies[20,21] have demonstrated that the resistance of tumor cells to reactive oxygen species may be one of the mechanisms of drug resistance.

Huber et al[22] found that crizotinib, with a dual inhibitory effect on hepatocyte growth factor receptor (HGFR, c-MET) and anaplastic lymphoma kinase (ALK) (Crizotinib), has a highly affinity with hMTH1. Crizotinib was approved by FDA in 2011 for the treatment of non-small cell lung cancer with positive echinoderm microtubule associated protein like 4-anaplastic lymphoma kinase (EML4-ALK)[23]. Moreover, S-Crizotinib is capable of inducing DNA damage of tumor cells and further inhibiting proliferation of tumor cells.

Previous studies have shown that hMTH1 inhibitors could selectively kill tumor cells but have little effect on normal cells. HMTH1 may be an effective target for treating tumors.

Therefore, we further investigated the effect of S-Crizotinib on parotid adenoma cell line ACC-M. The data showed that S-Crizotinib inhibited proliferation and induced apoptosis of

ACC-M cells. Meanwhile, SCGE results also showed that inhibited hMTH1 could lead to DNA damage in ACC-M cells.

Gad et al[8] reported that increased 8-oxodG, cell death and DNA repair mediated by ATM-p53 were observed in tumor cells treated with hMTH1 inhibitor[24]. Other key protective factors, such as p53, mutated in ataxia telangiectasia (ATM), ATR and RAS did not affect the roles of hMTH1 inhibitors, suggesting that hMTH1 inhibitors can still have an effect on tumor cells without these protective factors[25].

In summary, hMTH1 expression was up-regulated in patients with parotid adenoma, which may be helpful to monitor the disease condition. More importantly, hMTH1 is expected to serve as a therapeutic target for invasive parotid adenoma, especially in recurrent patients.

Conclusions

We observed that HMTH1 expression levels in patients with parotid adenoma and recurrent patients were remarkably higher than those in normal controls, which were positively correlated with the malignancy. Meanwhile, S-Crizotinib inhibited proliferation and induced apoptosis as well as DNA damage of ACC -M cells.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Cai Z, Chen H, Tao J, Guo W, Liu X, Zheng B, Sun W, Wang Y. Association of base excision repair gene polymorphisms with ESRD risk in a Chinese population. Oxid Med Cell Longev 2012; 2012: 928421.
2) Sheng Z, Oka S, Tsuchimoto D, Abolhassani N, Nomaru H, Sakumi K, Yamada H, Nakabeppu Y. 8-Oxoguanine causes neurodegeneration during MUTYH-mediated DNA base excision repair. J Clin Invest 2012; 122: 4344-4361.
3) Jin ZL, Pei H, Xu YH, Yu J, Deng T. The SUMO-specific protease SENP5 controls DNA damage response and promotes tumorigenesis in hepatocellular carcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 3566-3573.
4) Nakabeppu Y, Tsuchimoto D, Yamaguchi H, Sakumi K. Oxidative damage in nucleic acids and Parkinson's disease. J Neurosci Res 2007; 85: 919-934.
5) Nakabeppu Y, Oka S, Sheng Z, Tsuchimoto D, Sakumi K. Programmed cell death triggered by nucleotide pool damage and its prevention by MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase. Mutat Res 2010; 703: 51-58.
6) Oka S, Ohno M, Tsuchimoto D, Sakumi K, Furuichi M, Nakabeppu Y. Two distinct pathways of cell death triggered by oxidative damage to nuclear and mitochondrial DNAs. EMBO J 2008; 27: 421-432.
7) Boiteux S, Radicella JP. The human OGG1 gene: structure, functions, and its implication in the process of carcinogenesis. Arch Biochem Biophys 2000; 377: 1-8.
8) Cordoba-Canero D, Roldan-Arjona T, Ariza RR. Arabidopsis ZDP DNA 3'-phosphatase and ARP endonuclease function in 8-oxoG repair initiated by FPG and OGG1 DNA glycosylases. Plant J 2014; 79: 824-834.
9) Yan Y, Chen X, Li T, Li M, Liang H. Association of OGG1 Ser326Cys polymorphism and pancreatic cancer susceptibility: evidence from a meta-analysis. Tumour Biol 2014; 35: 2397-2402.
10) Grin IR, Dianov GL, Zharkov DO. The role of mammalian NEIL1 protein in the repair of 8-oxo-7,8-dihydroadenine in DNA. FEBS Lett 2010; 584: 1553-1557.
11) Hori M, Satou K, Harashima H, Kamiya H. Suppression of mutagenesis by 8-hydroxy-2'-deoxyguanosine 5'-triphosphate (7,8-dihydro-8-oxo-2'-deoxyguanosine 5'-triphosphate) by human MTH1, MTH2, and NUDT5. Free Radic Biol Med 2010; 48: 1197-1201.
12) Kim JI, Park YJ, Kim KH, Kim JI, Song BJ, Lee MS, Kim CN, Chang SH. HOGG1 Ser326Cys polymorphism modifies the significance of the environmental risk factor for colon cancer. World J Gastroenterol 2003; 9: 956-960.
13) Nakabeppu Y. Cellular levels of 8-oxoguanine in either DNA or the nucleotide pool play pivotal roles in carcinogenesis and survival of cancer cells. Int J Mol Sci 2014; 15: 12543-12557.
14) Karahalil B, Kesimci E, Emerce E, Gumus T, Kanbak O. The impact of OGG1, MTH1 and MnSOD gene polymorphisms on 8-hydroxy-2'-deoxyguanosine and cellular superoxide dismutase activity in myocardial ischemia-reperfusion. Mol Biol Rep 2011; 38: 2427-2435.
15) Sevilya Z, Leitner-Dagan Y, Pinchev M, Kremer R, Elinger D, Rennert HS, Schechtman E, Freedman LS, Rennert G, Paz-Elizur T, Livneh Z. Low integrated DNA repair score and lung cancer risk. Cancer Prev Res (Phila) 2014; 7: 398-406.
16) Borrego S, Vazquez A, Dasi F, Cerda C, Iradi A, Tormos C, Sanchez JM, Bagan L, Boix J, Zaragoza C, Camps J, Saez G. Oxidative stress and DNA dam-

age in human gastric carcinoma: 8-Oxo-7'8-dihydro-2'-deoxyguanosine (8-oxo-dG) as a possible tumor marker. Int J Mol Sci 2013; 14: 3467-3486.

17) Buchholz M, Braun M, Heidenblut A, Kestler HA, Kloppel G, Schmiegel W, Hahn SA, Luttges J, Gress TM. Transcriptome analysis of microdissected pancreatic intraepithelial neoplastic lesions. Oncogene 2005; 24: 6626-6636.

18) Patel A, Burton DG, Halvorsen K, Balkan W, Reiner T, Perez-Stable C, Cohen A, Munoz A, Giribaldi MG, Singh S, Robbins DJ, Nguyen DM, Rai P. MutT Homolog 1 (MTH1) maintains multiple KRAS-driven pro-malignant pathways. Oncogene 2015; 34: 2586-2596.

19) Speina E, Arczewska KD, Gackowski D, Zielinska M, Siomek A, Kowalewski J, Olinski R, Tudek B, Kusmierek JT. Contribution of hMTH1 to the maintenance of 8-oxoguanine levels in lung DNA of non-small-cell lung cancer patients. J Natl Cancer Inst 2005; 97: 384-395.

20) Gad H, Koolmeister T, Jemth AS, Eshtad S, Jacques SA, Strom CE, Svensson LM, Schultz N, Lundback T, Einarsdottir BO, Saleh A, Gokturk C, Baranczewski P, Svensson R, Berntsson RP, Gustafsson R, Stromberg K, Sanjiv K, Jacques-Cordonnier MC, Desroses M, Gustavsson AL, Olofsson R, Johansson F, Homan EJ, Loseva O, Brautigam L, Johansson L, Hoglund A, Hagenkort A, Pham T, Altun M, Gaugaz FZ, Vikingsson S, Evers B, Henriksson M, Vallin KS, Wallner OA, Hammarstrom LG, Wiita E, Almlof I, Kalderen C, Axelsson H, Djureinovic T, Puigvert JC, Haggblad M, Jeppsson F, Martens U, Lundin C, Lundgren B, Granelli I, Jensen AJ, Artursson P, Nilsson JA, Stenmark P, Scobie M, Berglund UW, Helleday T. MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool. Nature 2014; 508: 215-221.

21) Ohno M, Miura T, Furuichi M, Tominaga Y, Tsuchimoto D, Sakumi K, Nakabeppu Y. A genome-wide distribution of 8-oxoguanine correlates with the preferred regions for recombination and single nucleotide polymorphism in the human genome. Genome Res 2006; 16: 567-575.

22) Huber EM, Groll M. Inhibitors for the immuno- and constitutive proteasome: Current and future trends in drug development. Angew Chem Int Ed Engl 2012; 51: 8708-8720.

23) Streib M, Kraling K, Richter K, Xie X, Steuber H, Meggers E. An organometallic inhibitor for the human repair enzyme 7,8-dihydro-8-oxoguanosine triphosphatase. Angew Chem Int Ed Engl 2014; 53: 305-309.

24) Toledo LI, Murga M, Fernandez-Capetillo O. Targeting ATR and Chk1 kinases for cancer treatment: a new model for new (and old) drugs. Mol Oncol 2011; 5: 368-373.

25) Dominissini D, He C. Cancer: damage prevention targeted. Nature 2014; 508: 191-192.