Biochemical and Biophysical Research Communications 449 (2014) 49–54

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MET inhibitor PHA-665752 suppresses the hepatocyte growth factor-induced cell proliferation and radioresistance in nasopharyngeal carcinoma cells

Tongxin Liu [a,1], Qi Li [b,1], Quanquan Sun [a], Yuqin Zhang [a], Hua Yang [a], Rong Wang [a], Longhua Chen [a], Wei Wang [a,*]

[a] Department of Radiation Oncology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, PR China
[b] Guangdong Provincial Key Laboratory of Gastroenterology, Department of Gastroenterology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, PR China

ARTICLE INFO

Article history:
Received 11 April 2014
Available online 4 May 2014

Keywords:
Nasopharyngeal carcinoma
MET inhibitor
Radiosensitivity

ABSTRACT

Although ionizing radiation (IR) has provided considerable improvements in nasopharyngeal carcinoma (NPC), in subsets of patients, radioresistance is still a major problem in the treatment. In this study, we demonstrated that irradiation induced MET overexpression and activation, and the aberrant MET signal mediated by hepatocyte growth factor (HGF) induced radioresistance. We also found that MET inhibitor PHA-665752 effectively suppressed HGF induced cell proliferation and radioresistance in NPC cells. Further investigation indicated that PHA-665752 suppressed the phosphorylation of the Akt, ERK1/2, and STAT3 proteins in a dose-dependent manner. Our data indicated that the combination of IR with a MET inhibitor, such as PHA-665752, might be a promising therapeutic strategy for NPC.

1. Introduction

Nasopharyngeal carcinoma (NPC) is the most common cancer of the head and neck, and it is highly prevalent and endemic in Southern China and Southeast Asia, with an annual incidence rate of approximately 20 cases per 100,000 people in endemic areas [1]. The epidemiological evidence implies that environmental risk factors, Epstein-Barr virus (EBV) infection and genetic susceptibility play crucial roles in the carcinogenesis of NPC [2]. Whereas radiation therapy (RT) has remained the mainstay of treatment for early stage NPC, after primary treatment with radiotherapy or chemo-irradiation, more than 30% of patients will relapse with local or regional recurrence or distant metastasis [3]. Concurrent or palliative chemotherapy is given with radiotherapy for the treatment of locally advanced disease, such as stage III and non-metastatic stage IV disease [4,5]. The overall survival after recurrence is poor, with median survival ranging from 7.2 to 22 months [6–8]. Because of the advent of molecular targeted therapy over the past decade, some reagents such as the anti-EGFR antibody, cetuximab, and the anti-VEGF antibody, bevacizumab, have been employed against NPC [9,10]. Increasing evidence has shown that the combination of molecular target reagents and radiation might provide a more effective therapeutic strategy for NPC. However, the influence of molecular targeted therapy to the radiosensitivity of NPC has rarely been investigated.

MET, a transmembrane receptor tyrosine kinase, consists of a 45-kD a and a 150-kD subunit [11]. HGF, the only known ligand, binds to the extracellular region, leading to receptor dimerization and phosphorylation of the intracellular tyrosine kinase domains [11]. The activation/phosphorylation of the intracellular domain prompts activation of downstream signaling, predominantly through the phosphatidylinositide 3-kinase (PI3K)/Akt, mitogen-activated protein kinase (MAPK), and signal transducers and activators of the transcription (STAT) pathways [11–14]. MET has important roles in the malignant transformation of cancer cells [11]. Targeting MET has shown promise for the treatment of various types of cancer and is currently being tested in phase III trials for non-small cell lung cancer (NSCLC). In NPC, high MET expression was statistically significant for negative prognostic factors of survival [15].

Many studies have shown that MET is an attractive candidate for targeted anticancer therapy, frequently in combination with chemotherapy [16]. MET plays important roles in cancer metastasis and DNA damage and repair pathways [17,18]. Studies have shown that MET was involved in the radioresistance of tumors such as prostate cancer and brain metastasis of breast cancer

* Corresponding author.
E-mail address: wangwei9500@hotmail.com (W. Wang).
[1] These authors equally contributed to this work.

http://dx.doi.org/10.1016/j.bbrc.2014.04.147
0006-291X/

[19,20]. Ionizing radiation induces overexpression and activity of the MET oncogene, which promotes cell invasion and protects cells from apoptosis, thus supporting radioresistance [21]. Since the radiotherapy is essential in the treatments of NPC, the role of MET inhibition to the radiosensitivity of NPC should be evaluated specifically.

In this study, we investigated the anti-tumor and radiation-sensitizing effects of MET inhibitor PHA-665752 in NPC cells. Our findings suggested that the combination of MET inhibition and radiation might lead to important clinical benefits in NPC and provide the basis for further development of a targeted therapeutic strategy for NPC.

2. Materials and methods

2.1. Cell culture and reagents

The NPC cell line CNE-2 was kindly provided by Prof. Xia Yunfei (Sun Yat-sen University Cancer Center, Guangzhou, China). The CNE-1, HONE-1, SUNE-1 and NP69 cell lines were maintained in our lab. The human immortalized nasopharyngeal epithelial cell line NP69 was cultured in Keratinocyte/serum-free medium (Invitrogen, Carlsbad, CA, USA) containing bovine pituitary extract (BD biosciences, San Jose, CA, USA). The other human NPC cell lines were maintained in RPMI-1640 containing 10% fetal bovine serum (FBS), 100 units/ml penicillin, 50 μg/ml streptomycin and 2 mM of glutamine. All cell lines were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air.

The PHA-665752 (MET inhibitor) was obtained from Selleck Chemicals (Houston, TX, USA) and dissolved in dimethyl sulfoxide (DMSO, Sigma–Aldrich, St. Louis, MO, USA) at a concentration of 10 mM.

2.2. Cell proliferation assay

Cell proliferation assay was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium (MTT) dye reduction method [22]. Briefly, cells were seeded at a density of 2×10^3 cells/well in 96-well plates and incubated for 24 h. The cells were then treated with DMSO as a control or different concentrations of PHA-665752 and/or HGF (20 ng/ml). After 72 h treatment, 50 μl of MTT (5 mg/ml; Sigma–Aldrich) were added to the cell culture and incubated for 2 h at 37 °C. The reaction was stopped by the addition of 100 μl DMSO (Sigma–Aldrich). The absorbance was measured at 570 nm with a microplate reader. Results were expressed as a percentage of untreated group. Each experiment was performed independently at least three times, each with triplicate samples.

2.3. Antibodies and Western blotting

The primary antibodies used in this study were rabbit anti-MET (8198), phospho-MET (3135), Akt (9272), phospho-Akt (4060), STAT3 (4904), phospho-STAT3(4093), Erk1/2 (4695) and phospho-Erk1/2 (4094) (1:1000 each; Cell Signaling Technology, Danvers, MA). Western blotting was performed as previously described [23].

2.4. Clonogenic survival assays

CNE-1 and HONE-1 cells were seeded onto 60-mm dishes at specific cell densities. After overnight incubation, NPC cells were pretreated with DMSO as a vehicle control or PHA-665752 (1 μM) and/or HGF (20 ng/ml) for 1 h followed by irradiation to a dose of 0–8 Gy and washed away 24 h after irradiation. After 10–14 days, the cells were fixed in 100% methanol and stained with 0.5% crystal violet. Then, the colonies of at least 50 normal-appearing cells were counted and the survival fraction was calculated as described previously [24]. The data were analyzed using the single-hit multi-target model [25]. By using GraphPad Prism 5.0 software (GraphPad, La Jolla, CA), the survival fractions (SF) to fitted to the following single-hit multi-target formula: $SF = 1 - (1 - e^{-D/D0})^N$.

2.5. γ-H2AX assay

Residual DNA damage in irradiated CNE-1 and HONE-1 cells was determined by measuring residual γ-H2AX foci. The γ-H2AX antibody was used to visualize the dsDNA breaks, as previously described [26]. Briefly, 2×10^4 cells were plated in chamber slides and allowed to attach overnight. Then, the cells were pretreated with PHA-665752 (1 μM) and/or HGF (20 ng/ml) for 1 h before irradiation (4 Gy), and incubation for another 24 h. After that, the cells were fixed in 4% paraformaldehyde and incubated with a primary antibody against γ-H2AX (Abcam, San Francisco, CA, USA) overnight. Next, the primary antibody was washed off, and a secondary antibody conjugated to fluorescein isothiocyanate (FITC) was applied to the slides. Slides were examined using a fluorescence microscope (Olympus BX51, Tokyo, Japan). For each treatment condition, the γ-H2AX foci were counted in at least 50 cells.

2.6. Statistical analysis

Numerical results are expressed as the mean ± standard deviations (SD). The significant differences between the means were measured by the two-tailed unpaired student's *t*-test or one-way ANOVA. For all statistical analysis, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Irradiation induces overexpression and activation of MET in NPC cells

We first determined the expression levels of the total and phosphorylated MET in four NPC cell lines and NP69 cells. As shown in Fig. 1A, the expression of the total and phosphorylated MET was upregulated in NPC cell lines compared with NP69 cells, indicating a high level of activation of the MET pathway in NPC cells. Then, we assessed the effect of irradiation on the activation and expression of MET in the NPC cells. We selected CNE-1 and HONE-1 cells for this study and further investigation according to our preliminary experiments, as these two cell lines were more sensitive to the MET inhibitor PHA-665752. As shown in Fig. 1B, IR significantly increased the expression of MET and phospho-MET with two peaks after 1 h and 24 h in the CNE-1 and HONE-1 cells. These results demonstrated that irradiation induces overexpression and activation of MET in NPC cells.

3.2. HGF enhances cell proliferation by activating MET, which can be reversed by MET inhibitor PHA-665752

We next examined the effect of the MET ligand, HGF, on the proliferation of NPC cells. As shown in Fig. 2A, HGF strongly stimulated MET and increased the expression levels of phospho-MET as well as the total MET in NPC cells. Moreover, HGF significantly increased the proliferation of these cells (Fig. 2B). A MET tyrosine kinase inhibitor, PHA-665752 completely canceled the effect of

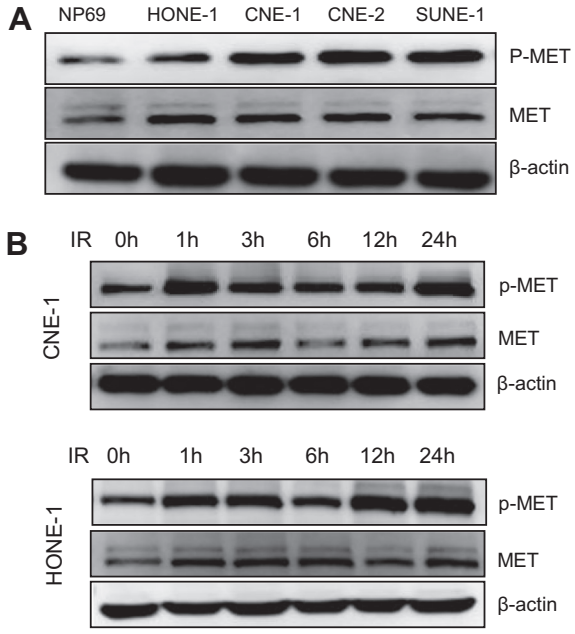

Fig. 1. Irradiation induces overexpression and activation of MET in NPC cells. (A) Basal expression of total and phosphorylated MET in nasopharyngeal carcinoma cell lines compared with an immortalized human nasopharyngeal epithelial cell line, NP69. (B) CNE-1 and HONE-1 cells were exposed to 4 Gy irradiation. The cell lysates were harvested at various time points after irradiation, and the indicated proteins were determined by Western blotting.

HGF (Fig. 2B). Further experiments showed that PHA-665752 inhibited cell proliferation in a dose-dependent manner in the CNE-1 and HONE-1 cells (Fig. 2C). These data suggested that the HGF/MET signaling pathway is critical for cell survival in NPC cells and that the MET tyrosine kinase inhibitor PHA-665752 could effectively block this pathway.

3.3. PHA-665752 increases the radiosensitivity and reverses the radioresistance induced by HGF in NPC cells

To evaluate the effect of MET inhibitor PHA-665752 on the radioresistance induced by HGF in NPC cells, clonogenic survival assay was performed. As shown in Fig. 3A, HGF increased the survival fraction of NPC cells after IR, whereas the PHA-665752 treatment significantly reduced the survival fraction and reversed the effect of HGF on the radiation response in NPC cells.

To further investigated the molecular mechanism of the radiosensitivity effect of MET inhibition, we tested the effects of PHA-665752 on the DNA damage response by measuring the number of γ-H2AX foci after irradiation, which is considered a sensitive molecular marker of DNA double strand breakage and repair. As shown in Fig. 3B, CNE-1 and HONE-1 cells treated with PHA-665752 in combination with IR led to a dramatic persistence of the γ-H2AX foci at 24 h post-IR administration compared with exposure to IR alone. Cells treated with HGF and IR led to a less persistence of γ-H2AX foci compared with exposure to IR alone, whereas the administration of PHA-665752 reversed the effect induced by HGF. These results clearly demonstrated that MET inhibitor PHA-665752 increases the radiosensitivity and reverses the radioresistance induced by HGF in NPC cells in a manner that might be associated with the persistence of DNA damage.

3.4. PHA-665752 induces suppression of three downstream signaling pathways in NPC cells

Next, we examined which downstream pathway(s) was responsible for the effect of the MET inhibitor PHA-665752 in the NPC cells. As shown in Fig. 4, we found that PHA-665752 inhibited the phosphorylation of MET in the CNE-1 and HONE-1 cells, thereby slightly inhibiting the phosphorylation of Akt, ERK1/2 and STAT3. Although PHA-665752 strongly inhibited the MET phosphorylation even at a low concentration (0.1 μM), the phosphorylation of the downstream signaling molecules of these three

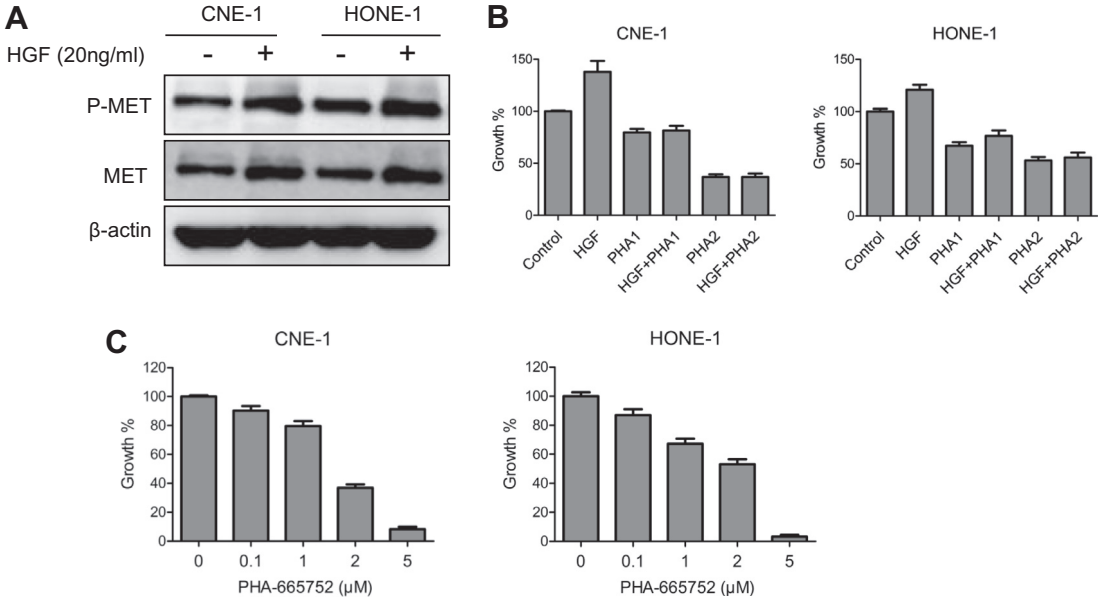

Fig. 2. HGF enhances cell proliferation by activating MET, which can be reversed by MET inhibitor PHA-665752. (A) CNE-1 and HONE-1 cells were incubated with 20 ng/ml of HGF for 1 h. The cell lysates were harvested and the indicated proteins were determined by Western blotting. (B) CNE-1 and HONE-1 cells were incubated with HGF (20 ng/ml) and/or PHA-665752, and the cell growth was determined after 72 h using an MTT assay. Data are presented as the mean ± SD. The bars indicate the SD. PHA1, 1 μM of PHA-665752; PHA2, 2 μM of PHA-665752. $^{*}P < 0.05$, compared with control group. (C) CNE-1 and HONE-1 cells were incubated with various concentrations of PHA-665752, and the cell growth was determined after 72 h using an MTT assay. $^{*}P < 0.05$, compared with control group.

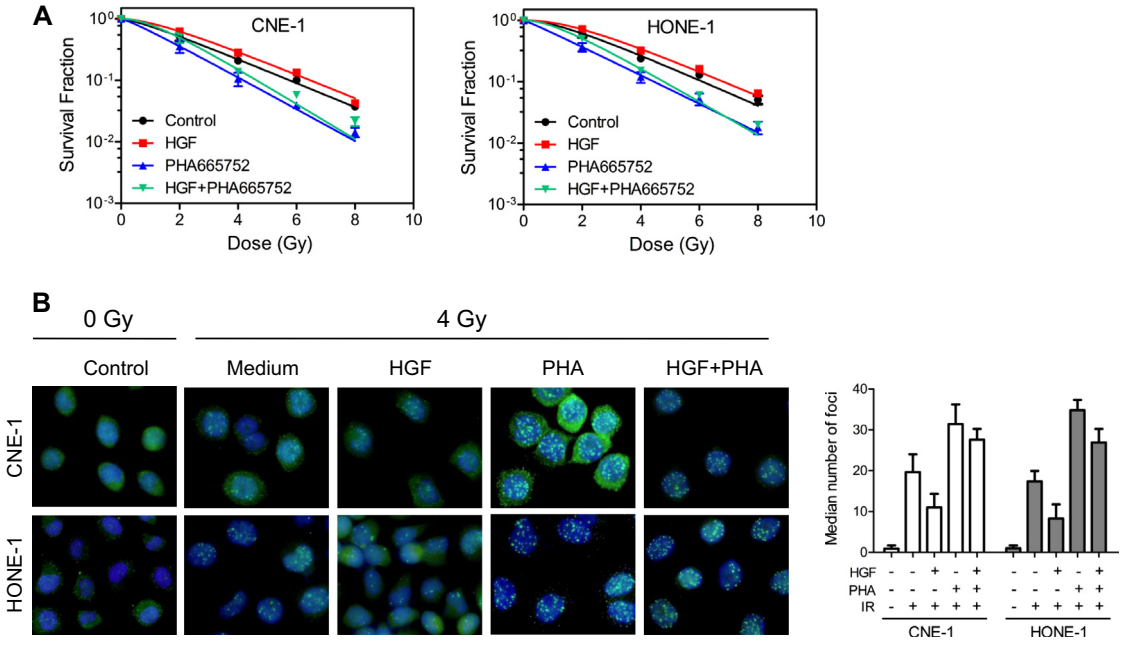

Fig. 3. PHA-665752 increases the radiosensitivity and reverses the radioresistance induced by HGF in NPC cells. (A) The clonogenic survival assays of the CNE-1 and HONE-1 cells treated with PHA-665752 (1 μM) and/or HGF (20 ng/ml) followed by irradiation in a range of radiation doses. Each data point represents the mean of three experiments ± SD. The bars indicate the SD. (B) Radiosensitization induced by PHA-665752 is accompanied by the persistence of the γ-H2AX foci. (Left) Representative immunofluorescence micrographs of the γ-H2AX foci formation in PHA-665752 (1 μM), HGF (20 ng/ml), IR and their combination groups. The micrographs were taken at ×400 magnification. (Right) The median number of the γ-H2AX foci per cell. The bars indicate the SD. $^{*}P < 0.01$ compared with the IR alone group.

pathways were suppressed slightly. The phosphor-ERK1/2 was significantly inhibited by PHA-665752 in the CNE-1 cells, whereas a similar level of inhibition was not found in the HONE-1 cells, although PHA-665752 showed similar effects in both cell lines in cell growth inhibition and radiosensitization. These data suggested that the PHA-665752 inhibition of cell proliferation and the increase in the radiosensitivity of NPC cells might not be through a single downstream pathway.

4. Discussion

In this study, we demonstrated that IR induced the overexpression and activation of MET in NPC cells. We also found that HGF stimulated the proliferation and radioresistance of NPC cells by activating MET, which could be overcome by MET inhibitor PHA-665752. Further investigation indicated that PHA-665752 suppressed the phosphorylation of the Akt, ERK1/2, and STAT3 proteins in a dose-dependent manner. Our results suggest that the MET inhibitor PHA-665752 could effectively sensitizes NPC cells to radiation.

Over the last decade, combination of chemotherapy and radiotherapy for recurrent or metastatic NPC has led to the increase in response rates, which also accompanied by unavoidable increased toxicity. Various biological agents are directed at different therapeutic targets, such as the EGFR, the VEGFR and other potential targets. Cetuximab is the only biological agent in its class that appears to be a promising agent to restore chemo-sensitivity in heavily pretreated NPC patients, as well as in combination with standard chemo-irradiation. In a phase II study using a combination of cetuximab with cisplatin and intensity-modulated radiotherapy (IMRT) in patients with stage III or stage IV NPC, the two-year pro-

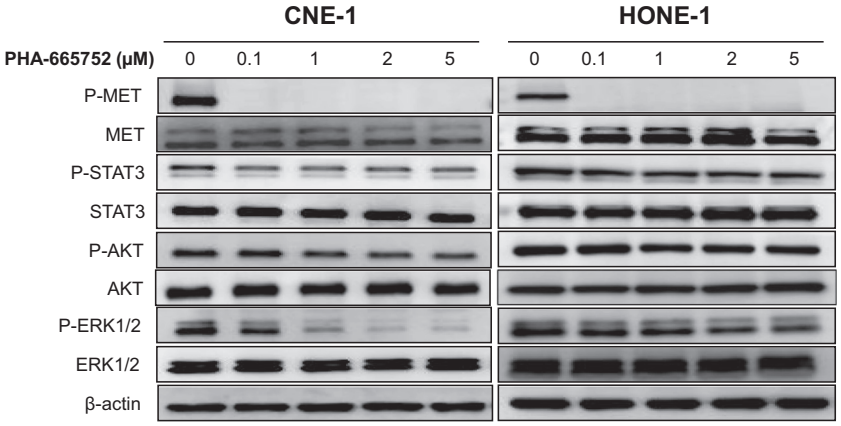

Fig. 4. PHA-665752 induces suppression of three downstream signaling pathways in NPC cells. CNE-1 and HONE-1 cells were incubated with various concentrations of PHA-665752 for 1 h. The cell lysates were harvested and the phosphorylation of the indicated proteins was determined by Western blotting.

gression free survival (PFS) was 86.5%. In this clinical study, the patients had a high incidence of grade 3–4 mucositis (87%) and grade 3 radiotherapy-related dermatitis (20%) [9]. This result indicated that although IMRT decreased the side effects of radiotherapy for NPC when combined therapies with biological agents were used, the side effects remained a problem. The prevention of radiation side effect includes ensuring the radiosensitization of cancer cells and the radioprotection of normal cells. MET activity has been associated with increased radiation resistance. MET is an upstream activator of Akt, which has been linked to radiation resistance [27]. Because MET was higher expressed and activated in NPC cells than related normal cells, MET inhibition could be a good candidate for decreasing the side effects of radiation. In this study, our data showed that a MET inhibitor PHA-665752 significantly inhibited cell proliferation as well as increased radiosensitivity. A combination of PHA-665752 and irradiation might be an ideal strategy to decrease the dose of radiotherapy and hence minimize the side effects of radiotherapy.

Recent studies have demonstrated that increasing DNA damage has emerged as an attractive path to enhance tumor cell radiosensitivity [28]. Our data indicated that the radiosensitization effect of PHA-665752 on the NPC cells was associated with an increased persistence of the γ-H2AX foci after IR, suggesting that the cellular DNA damage increased upon the MET inhibition. Although the rationale for the MET inhibition to increase the DNA damage ability has not been well documented, it has been reported that the signaling pathways downstream of RTKs intersect with the DNA damage mechanisms to modulate a cellular response to IR. All of the MAPK/PI3K/STAT pathways were reported to be involved in DNA damage and repair [29,30]. Our results showed that the selective MET inhibitor, PHA-665752, could efficiently inhibit the phosphorylation of AKT, MAPK, and STAT3, which are three key components of the MET signaling pathways, and regulate the DNA damage and repair ability in NPC cells.

We have shown that HGF stimulated the proliferation and radioresistance of NPC cells by activating the MET. High HGF expression in tumor cells or in stromal cells was significantly associated with an advanced clinical stage, lymph node metastasis, and a worse prognosis of NPC patients [31,32]. Hypoxia is a common reason for radioresistance. The HGF/MET signal pathway is reported to be activated by hypoxia and to result in migration and metastasis [33]. The HGF/MET signal might be involved in hypoxia-induced radioresistance in NPC. MET inhibition might radiosensitize NPC by deactivating the downstream signals as well as reducing the hypoxia-induced radioresistance. This finding should be evaluated in a further study.

Our results showed that targeting HGF/MET signaling in NPC has anti-tumor activity and improves the efficacy of radiotherapy. The radiosensitizing effect of the MET inhibitor, PHA-665752, might be caused by increasing the residual DNA damage. These effects were due to the simultaneous inhibition of three downstream signaling pathways in NPC cells. These findings suggest that a combination of IR and PHA-665752 might be a useful therapeutic strategy against NPC.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We are grateful to Prof. Xia Yunfei for providing the NPC cell lines. This study was supported by a National Natural Science Foundation of China Grant (W. Wang, 81172243; and Q. Li, 81201961).

References

[1] H.H. Loong, B.B. Ma, A.T. Chan, Update on the management and therapeutic monitoring of advanced nasopharyngeal cancer, Hematol. Oncol. Clin. North Am. 22 (2008) 1267–1278, x.
[2] W.I. Wei, J.S. Sham, Nasopharyngeal carcinoma, Lancet 365 (2005) 2041–2054.
[3] A.W. Lee, W.M. Sze, J.S. Au, S.F. Leung, T.W. Leung, D.T. Chua, B.C. Zee, S.C. Law, P.M. Teo, S.Y. Tung, D.L. Kwong, W.H. Lau, Treatment results for nasopharyngeal carcinoma in the modern era: the Hong Kong experience, Int. J. Radiat. Oncol. Biol. Phys. 61 (2005) 1107–1116.
[4] E.E. Vokes, D.N. Liebowitz, R.R. Weichselbaum, Nasopharyngeal carcinoma, Lancet 350 (1997) 1087–1091.
[5] H. Ali, M. Al-Sarraf, Chemotherapy in advanced nasopharyngeal cancer, Oncology (Williston Park) 14 (2000) 1223–1230 (discussion 1232-7, 1239-42).
[6] Y. Bensouda, W. Kaikani, N. Ahbeddou, R. Rahhali, M. Jabri, H. Mrabti, H. Boussen, H. Errihani, Treatment for metastatic nasopharyngeal carcinoma, Eur. Ann. Otorhinolaryngol. Head Neck Dis. 128 (2011) 79–85.
[7] F. Caponigro, F. Longo, F. Ionna, F. Perri, Treatment approaches to nasopharyngeal carcinoma: a review, Anticancer Drugs 21 (2010) 471–477.
[8] B.B. Ma, A.T. Chan, Systemic treatment strategies and therapeutic monitoring for advanced nasopharyngeal carcinoma, Expert Rev. Anticancer Ther. 6 (2006) 383–394.
[9] S.L. Chan, B.B. Ma, Novel systemic therapeutic for nasopharyngeal carcinoma, Expert Opin. Ther. Targets 16 (Suppl. 1) (2012) S63–S68.
[10] N.Y. Lee, Q. Zhang, D.G. Pfister, J. Kim, A.S. Garden, J. Mechalakos, K. Hu, Q.T. Le, A.D. Colevas, B.S. Glisson, A.T. Chan, K.K. Ang, Addition of bevacizumab to standard chemoradiation for locoregionally advanced nasopharyngeal carcinoma (RTOG 0615): a phase 2 multi-institutional trial, Lancet Oncol. 13 (2012) 172–180.
[11] C.R. Maroun, T. Rowlands, The Met receptor tyrosine kinase: A key player in oncogenesis and drug resistance, Pharmacol. Ther. 142 (2014) 316–338.
[12] C. Birchmeier, W. Birchmeier, E. Gherardi, W.G. Vande, Met, metastasis, motility and more, Nat. Rev. Mol. Cell Biol. 4 (2003) 915–925.
[13] Q. Zeng, S. Chen, Z. You, F. Yang, T.E. Carey, D. Saims, C.Y. Wang, Hepatocyte growth factor inhibits anoikis in head and neck squamous cell carcinoma cells by activation of ERK and Akt signaling independent of NFkappa B, J. Biol. Chem. 277 (2002) 25203–25208.
[14] R.M. Anderson, M. Delous, J.A. Bosch, L. Ye, M.A. Robertson, D. Hesselson, D.Y. Stainier, Hepatocyte growth factor signaling in intrapancreatic ductal cells drives pancreatic morphogenesis, PLoS Genet. 9 (2013) e1003650.
[15] Y.J. Kim, H. Go, H.G. Wu, Y.K. Jeon, S.W. Park, S.H. Lee, Immunohistochemical study identifying prognostic biomolecular markers in nasopharyngeal carcinoma treated by radiotherapy, Head Neck 33 (2011) 1458–1466.
[16] Y.J. Kim, J.S. Choi, J. Seo, J.Y. Song, S.E. Lee, M.J. Kwon, M.J. Kwon, J. Kundu, K. Jung, E. Oh, Y.K. Shin, Y.L. Choi, MET is a potential target for use in combination therapy with EGFR inhibition in triple-negative/basal-like breast cancer, Int. J. Cancer 134 (2014) 2424–2436.
[17] O.O. Ogunwobi, W. Puszyk, H.J. Dong, C. Liu, Epigenetic upregulation of HGF and c-Met drives metastasis in hepatocellular carcinoma, PLoS One 8 (2013) e63765.
[18] V. Bhardwaj, T. Cascone, M.A. Cortez, A. Amini, J. Evans, R.U. Komaki, J.V. Heymach, J.W. Welsh, Modulation of c-Met signaling and cellular sensitivity to radiation: potential implications for therapy, Cancer 119 (2013) 1768–1775.
[19] H. Yang, H.W. Lee, Y. Kim, Y. Lee, Y.S. Choi, K.H. Kim, J. Jin, J. Lee, K.M. Joo, D.H. Nam, Radiosensitization of brain metastasis by targeting c-MET, Lab. Invest. 93 (2013) 344–353.
[20] H. Yu, X. Li, S. Sun, X. Gao, D. Zhou, C-Met inhibitor SU11274 enhances the response of the prostate cancer cell line DU145 to ionizing radiation, Biochem. Biophys. Res. Commun. 427 (2012) 659–665.
[21] F. De Bacco, P. Luraghi, E. Medico, G. Reato, F. Girolami, T. Perera, P. Gabriele, P.M. Comoglio, C. Boccaccio, Induction of MET by ionizing radiation and its role in radioresistance and invasive growth of cancer, J. Natl. Cancer Inst. 103 (2011) 645–661.
[22] L.M. Green, J.L. Reade, C.F. Ware, Rapid colorimetric assay for cell viability: application to the quantitation of cytotoxic and growth inhibitory lymphokines, J. Immunol. Methods 70 (1984) 257–268.
[23] S. Yano, W. Wang, Q. Li, K. Matsumoto, H. Sakurama, T. Nakamura, H. Ogino, S. Kakiuchi, M. Hanibuchi, Y. Nishioka, H. Uehara, T. Mitsudomi, Y. Yatabe, T. Nakamura, S. Sone, Hepatocyte growth factor induces gefitinib resistance of lung adenocarcinoma with epidermal growth factor receptor-activating mutations, Cancer Res. 68 (2008) 9479–9487.
[24] G. Xie, J. Zhan, Y. Tian, Y. Liu, Z. Chen, C. Ren, Q. Sun, J. Lian, L. Chen, J. Ruan, C. Ye, A. Sun, Y. Yuan, Mammosphere cells from high-passage MCF7 cell line show variable loss of tumorigenicity and radioresistance, Cancer Lett. 316 (2012) 53–61.
[25] B. Fertil, H. Dertinger, A. Courdi, E.P. Malaise, Mean inactivation dose: a useful concept for intercomparison of human cell survival curves, Radiat. Res. 99 (1984) 73–84.
[26] R. Prevo, E. Deutsch, O. Sampson, J. Diplexcito, K. Cengel, J. Harper, P. O'Neill, W.G. McKenna, S. Patel, E.J. Bernhard, Class I PI3 kinase inhibition by the pyridinylfuranopyrimidine inhibitor PI-103 enhances tumor radiosensitivity, Cancer Res. 68 (2008) 5915–5923.
[27] J. Brognard, A.S. Clark, Y. Ni, P.A. Dennis, Akt/protein kinase B is constitutively active in non-small cell lung cancer cells and promotes cellular survival and resistance to chemotherapy and radiation, Cancer Res. 61 (2001) 3986–3997.

[28] S. Bao, Q. Wu, R.E. McLendon, Y. Hao, Q. Shi, A.B. Hjelmeland, M.W. Dewhirst, D.D. Bigner, J.N. Rich, Glioma stem cells promote radioresistance by preferential activation of the DNA damage response, Nature 444 (2006) 756–760.
[29] A. Sallmyr, J. Fan, F.V. Rassool, Genomic instability in myeloid malignancies: increased reactive oxygen species (ROS), DNA double strand breaks (DSBs) and error-prone repair, Cancer Lett. 270 (2008) 1–9.
[30] R.E. Meyn, A. Munshi, J.V. Haymach, L. Milas, K.K. Ang, Receptor signaling as a regulatory mechanism of DNA repair, Radiother. Oncol. 92 (2009) 316–322.
[31] L.Q. Xie, L.J. Bian, Z. Li, Y. Li, Z.X. Li, B. Li, Altered expression of E-cadherin by hepatocyte growth factor and effect on the prognosis of nasopharyngeal carcinoma, Ann. Surg. Oncol. 17 (2010) 1927–1936.
[32] L.Q. Xie, L.J. Bian, Z. Li, Y. Li, Y.J. Liang, Co-elevated expression of hepatocyte growth factor and interleukin-8 contributes to poor prognosis of patients with primary nasopharyngeal carcinoma, Oncol. Rep. 23 (2010) 141–150.
[33] J.P. Eder, W.G. Vande, S.A. Boerner, P.M. LoRusso, Novel therapeutic inhibitors of the c-Met signaling pathway in cancer, Clin. Cancer Res. 15 (2009) 2207–2214.