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Protective autophagy is involved in resistance towards MET inhibitors in human gastric adenocarcinoma cells

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ABSTRACT

MET, also known as hepatocyte growth factor receptor (HGFR), is a receptor tyrosine kinase with an important role, both in normal cellular function as well as in oncogenesis. In many cancer types, abnormal activation of MET is related to poor prognosis and various strategies to inhibit its function, including small molecule inhibitors, are currently in preclinical and clinical evaluation. Autophagy, a self-digesting recycling mechanism with cytoprotective functions, is induced by cellular stress. This process is also induced upon cytotoxic drug treatment of cancer cells and partially allows these cells to escape cell death. Thus, since autophagy protects different tumor cells from chemotherapy-induced cell death, current clinical trials aim at combining autophagy inhibitors with different cancer treatments. We found that in a gastric adenocarcinoma cell line GTL-16, where MET activity is deregulated due to receptor overexpression, two different MET inhibitors PHA665752 and EMD1214063 lead to cell death paralleled by the induction of autophagy. A combined treatment of MET inhibitors together with the autophagy inhibitor 3-MA or genetically impairing autophagy by knocking down the key autophagy gene ATG7 further decreased cell viability of gastric cancer cells. In general, we observed the induction of cytoprotective autophagy in MET expressing cells upon MET inhibition and a combination of MET and autophagy inhibition resulted in significantly decreased cell viability in gastric cancer cells.

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1. Introduction

Signaling through MET, the tyrosine kinase receptor for hepatocyte growth factor (HGF), is crucial for embryonic development and the HGF–MET axis plays an important role in adult tissue regeneration. Moreover, MET is associated with anti-apoptotic, anti-inflammatory and angiogenic functions [1]. In tumorigenesis, the relevance of aberrant MET function in malignant cellular transformation and in the pathogenesis of human cancers has been firmly established. Deregulation of HGF/MET signaling is found in several types of human malignancies, including gastric carcinoma [2,3]. The aberrant MET activity in cancer is usually a result of MET gene overexpression or less frequently it is due to the presence of activating point mutations in MET [4]. MET overexpression with or without gene amplification is a prominent characteristic of several epithelial and mesenchymal cancers [5]. In gastric carcinoma cells, MET silencing inhibits the invasion program and decreases tumor growth [6] pointing to a role for deregulated MET in gastric tumor progression.

Cells expressing aberrantly activated MET can gain proliferative advantage but the inhibition of MET in a subset of those cells will induce cell death. Tumor cells that depend on the persistent activity of the MET oncogene for their continuous growth have been termed 'MET oncogene-addicted' cells [4,7]. The phenomenon of MET oncogene addiction was found in gastric carcinoma and non-small cell lung cancer (NSCLC) cell lines but also in primary tumors such as gastric and oesophagal carcinomas, colorectal cancer, NSCLC, medulloblastomas and glioblastomas [4]. Thus, MET-addicted tumors represent promising candidates for the treatment with specific MET inhibitors. Supporting this hypothesis, tyrosine kinase inhibitors (TKIs) including the two prototypic MET targeting small molecules SU11274 and PHA665752 developed by SUGEN (Pfizer) show promising results by selectively suppressing growth,

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migration and survival in various cancer cell lines as well as tumor growth in MET-driven xenograft models [5,8,9]. Furthermore, MET inhibitors increase tumor cytotoxicity when combined with DNA damaging agents indicating that therapeutic strategies combining MET inhibitors with additional genotoxic treatments are beneficial [10,11]. Currently, roughly 20 different small molecule TKIs of MET have been reported, several of which (e.g., PF-04217903 (Pfizer), EMD1214063 (Merck)) are at various stages of clinical trials [12]. As with other kinase inhibitors, one might anticipate that along with the routine use of MET inhibitors in the clinics, treatment failure due to the acquisition of resistance mechanisms will arise in some patients, similarly to the experience gained by using specific kinase inhibitors such as imatinib or erlotinib in CML and lung cancer, respectively [13,14]. Resistance might evolve due to a second mutation in the target gene or activation of other pathways to bypass the targeted pathway. On the other hand, tumor cells can activate survival pathways to escape drug-induced cell death, for example by inducing autophagy.

Autophagy (referring to macroautophagy) is a proteolytic self-degradation process. At base level activity it mainly functions as a homeostatic mechanism to prevent accumulation of defect metabolites and organelles. Autophagy is also induced as a response to cellular stress such as starvation allowing for prolonged cell survival. Thus, autophagy is mostly a prosurvival process with cytoprotective functions [15,16]. On the other hand, few reports point to a direct role for autophagy in caspase-independent cell death [17–19]. Furthermore, tumor cells seem to have a higher dependency on autophagy than normal cells due to increased stress caused by the unfavorable cellular environment [20]. Thus, drugs that inhibit autophagic activity may particularly target cancer cells [15,21]. Additionally, genotoxic cancer drugs frequently induce autophagy in tumor cells as a cytoprotective stress response to the induced damages. Therefore, blocking autophagy in combination with other treatments might enhance cytotoxicity [20]. Indeed, several ongoing clinical trials in patients with solid or hematopoietic tumors interrogate if inhibition of autophagy in combination with chemotherapy would be beneficial (reviewed in [20]).

We thus investigated if autophagy might be involved in resistance of gastric cancer cells to MET-inhibitors. We found that MET inhibition in gastric carcinoma cells caused a marked induction of autophagy. Importantly, inhibition of autophagy by chemical and genetic means in combination with MET-inhibitor treatment significantly enhanced cytotoxicity.

2. Materials and methods

2.1. Cell lines

Human gastric adenocarcinoma cell lines GTL-16 and MKN45 were obtained from Paolo Comoglio (Medical School University of Torino, Torino, Italy) and from Silvia Giordano (Torino, Italy), respectively. Both cell lines were grown in RPMI medium (GIBCO, Invitrogen Corp.) supplemented with 5% FCS (Sigma) and antibiotic–antimycotic (penicillin 100 U/ml, streptomycin sulfate 100 U/ml, amphotericin B as Fungizone 0.25 μg/ml; GIBCO).

2.2. Reagents

MET small molecule tyrosine kinase inhibitors PHA665752 (Pfizer, La Jolla, CA) and EMD1214063 (Merck, Darmstadt, Germany) were dissolved in DMSO and used at indicated concentrations. Bafilomycin A1 (dissolved in DMSO, Alexis biochemicals) was used at 50 nM, 3-methyladenine (3-MA dissolved in H_2O, Sigma–Aldrich) at 5 mM, Chloroquine (CQ dissolved in H_2O, Sigma–Aldrich) and lithium chloride (LiCl, dissolved in H_2O, Sigma–Aldrich) at 25 mM.

2.3. Western blotting

Whole cell extracts were prepared using RIPA lysis buffer supplemented with 8 M UREA according to the protocol given by www.abcam.com, and 40–60 μg protein was loaded on 10% denaturing polyacrylamide gels. Blots were incubated with the primary antibodies in TBS 0.05% Tween-20/5% milk overnight at 4 °C, incubated with secondary anti-mouse or -rabbit antibodies at 1:5–10,000 for 1 h at room temperature, and analyzed using the Infrared Fluorescent Imaging system Odyssey (LI-COR Biosciences). Primary antibodies used were anti-LC3B (Novus Biological) 1:500, anti-phospho-MET (Tyr1234/1235, Cell Signaling Technology) 1:1000, anti-ATG7 (Sigma) 1:500 and anti-GAPDH (Millipore) 1:5000.

2.4. Real-time quantitative reverse transcription-PCR (qPCR)

Total RNA was extracted using the RNeasy Mini Kit and the RNase-Free DNase Set according to the manufacturer's protocol (Qiagen, Hombrechtikon, Switzerland). Total RNA was reverse transcribed using random primers (Roche Diagnostics) and M-MLV reverse transcriptase (Promega). PCR and fluorescence detection were performed using the ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, Rotkreuz, Switzerland).

For quantification of ATG7 and LC3B *mRNA* Taqman® Gene Expression Assays Hs00197738_m1, and Hs00797944_m1, (Applied Biosystems) were used, respectively. HMBS primers and probes have been described [22]. *N*-fold changes were calculated using the $\Delta\Delta Ct$ method of relative quantification.

2.5. Lentiviral vectors

Lentiviral vectors expressing shRNAs targeting ATG7 were purchased from Sigma–Aldrich (SHGLY-NM_014326). An Cherry-LC3 lentiviral vector was kindly provided by Dr. Maria S. Soengas (CNIO, Molecular Pathology Program, Madrid, Spain). All vectors contain a puromycin resistance gene for selection of transduced mammalian cells. Lentivirus production and transduction were done as described [22]. Combinations of pLKO.1-puro lentiviral vectors targeting ATG7 NM_006395.1-491s1c1 (TRCN0000007585) and NM_006395.1-2173s1c1 (TRCN0000007584) were used to knockdown ATG7 in GTL-16 cells. Cell populations were selected with 1.5 μg/ml puromycin for 4 days and knock-down efficiency was assessed by qPCR.

2.6. Cell viability assay

Cells were seeded in flat-bottom 96-well culture plates at a density of 2×10^4 cells/well. Cells were treated with MET inhibitors (PHA 100 nM or 300 nM, EMD 10 nM or 50 nM) alone or in combination with 3-MA (5 mM), CQ (25 μM) or LiCl (25 nM). The plates were incubated at 37 °C and 5% CO_2 for 24 h. Ten microliters of Alamar Blue was then added to each well and the plates were incubated for 2 h before absorbance was measured at 590 nm and 630 nm. Growth medium was used as a reference (Abs0). Data were obtained using the following formulas: viability = Abs(590)−(Abs (630) × (Abs0(590) − Abs0(630))). Values were normalized to non-treated control cells.

2.7. Fluorescence microscopy

mCherry-LC3 expressing GTL-16 cells were treated with PHA665752 (300 nM) or EMD1214063 (50 nM). mCherry-LC3 dot

formation was detected using Nikon Eclipse E800 microscope (Nikon), magnification sections were viewed through a 10× eyepiece using a 60× objective (NA = 0.13, Nikon). Color balance and contrast were adjusted using Image J software.

2.8. Statistical analysis

Nonparametric Mann–Whitney-*U* tests were applied to compare the difference between two groups using Prism software. *p*-values <0.05 were considered to be statistically significant.

3. Results

3.1. MET inhibition induces autophagy in gastric cancer cells

Since autophagy is often induced upon cellular stress, we aimed at investigating if MET inhibition will modulate autophagy in GTL-16 gastric adenocarcinoma cells that overexpress the MET receptor. To this end, GTL-16 cells were treated with the specific MET small molecule inhibitors EMD1214063 (10 nM or 50 nM) or PHA665752 (100 nM or 300 nM). To evaluate the autophagic flux, co-treatment with Bafilomycin A1 (Baf A1) was performed during 24 h in order to block autophagy. Baf A1 prevents maturation of the autophagosome by inhibiting the fusion between the autophagic vacuole to lysosomes. The block in autophagic flux in the last step of autophagosome maturation will lead to accumulation of autophagosome in the cytoplasm if MET inhibition activates autophagy. Conversion of LC3-I to LC3-II as a measurement of autophagy induction was measured before and after the treatment by Western blotting. We observed an accumulation of LC3-II upon combined treatment of GTL-16 cells by MET inhibitor and Baf A1 and the conversion from LC3-I to LC3-II in single treatments with both MET inhibitors (Fig. 1A). A prolonged treatment with either EMD1214063 or PHA665752 (up to 72 h) revealed a continuous autophagic activity as determined by LC3 conversion (Fig. 1B). As additional markers for the activation of autophagy we measured the induction of LC3B and ATG7 mRNA levels. Both MET inhibitors induced LC3B and ATG7 transcription already after 24 h of treatment (Fig. 1C). To further validate our findings, GTL-16 cells were transduced with a mCherry-LC3 lentiviral vector. GTL-16 mCherry-LC3 cells were treated with MET inhibitors and subsequently the formation of mCherry-LC3 dots was evaluated by fluorescence microscopy. We were able to confirm the enhanced autophagic activity upon treatment with MET inhibitors by the occurrence of mCherry-LC3 dots in treated cells as compared to untreated cells (Fig. 1D). In addition, we observed a similar increase in autophagic activity after MET inhibition in a second gastric carcinoma cell line, namely MKN45 (Suppl. Fig. 1). Overall, our findings clearly

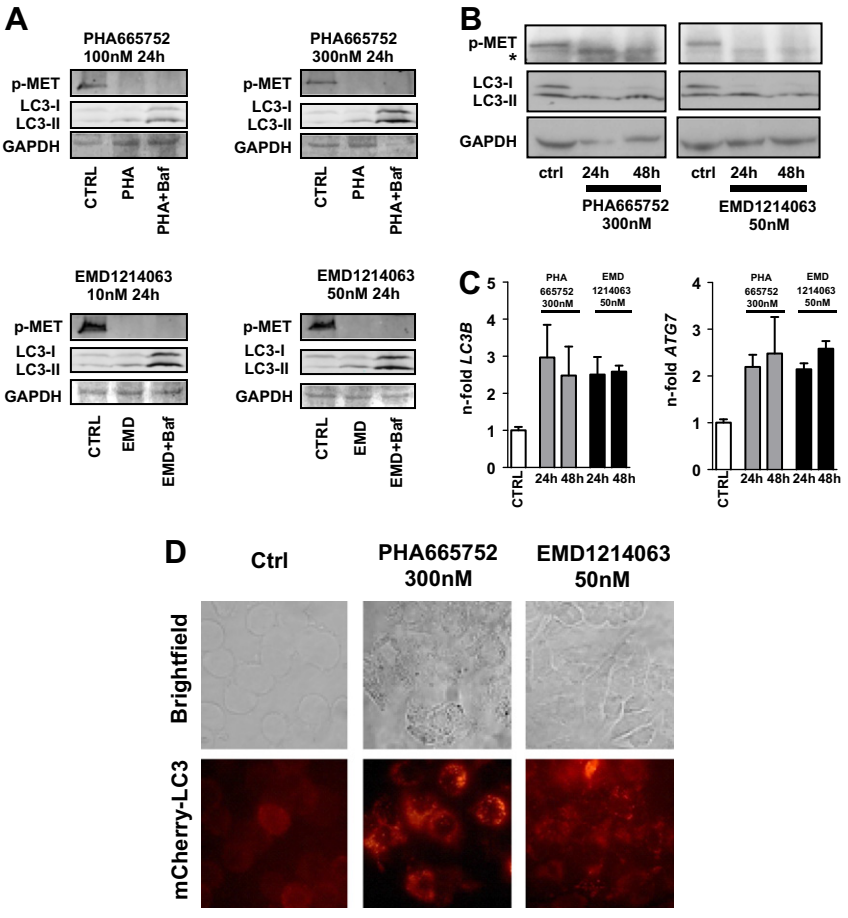

Fig. 1. MET inhibition induces autophagy in gastric carcinoma cells. (A) Western blot analysis of phospho-MET (Tyr1234/1235) and LC3B expression in GTL-16 cell line treated with the MET inhibitors EMD1214063 (10 nM or 50 nM) or PHA665752 (100 nM or 300 nM) and co-treated with Bafilomycin A1 for 24 h. Total protein was extracted from GTL-16 cells and submitted to immunoblotting using anti-phospho-MET (Tyr1234/1235) and anti-LC3B antibodies. GAPDH is shown as a loading control. (B) Time-course of autophagy induction upon MET inhibition. GTL-16 cells were treated with PHA665752 or EMD1214063 for 24 h and 48 h. Western blot analysis as in A. (C) ATG7 and LC3B mRNA expression levels in GTL-16 cells treated with EMD1214063 (50 nM) or PHA665752 (300 nM) for 24 h and 48 h. Values are normalized to the housekeeping gene *HMBS* and are given as *n*-fold increase relative to non-treated cells. (D) GTL-16 cells were transduced with mCherry-LC3 and were treated with EMD1214063 (50 nM) or PHA665752 (300 nM) for 24 h. Treated cells were fixed and mounted, and mCherry signals were analyzed by fluorescence microscopy. Representative images from two independent experiments are shown.

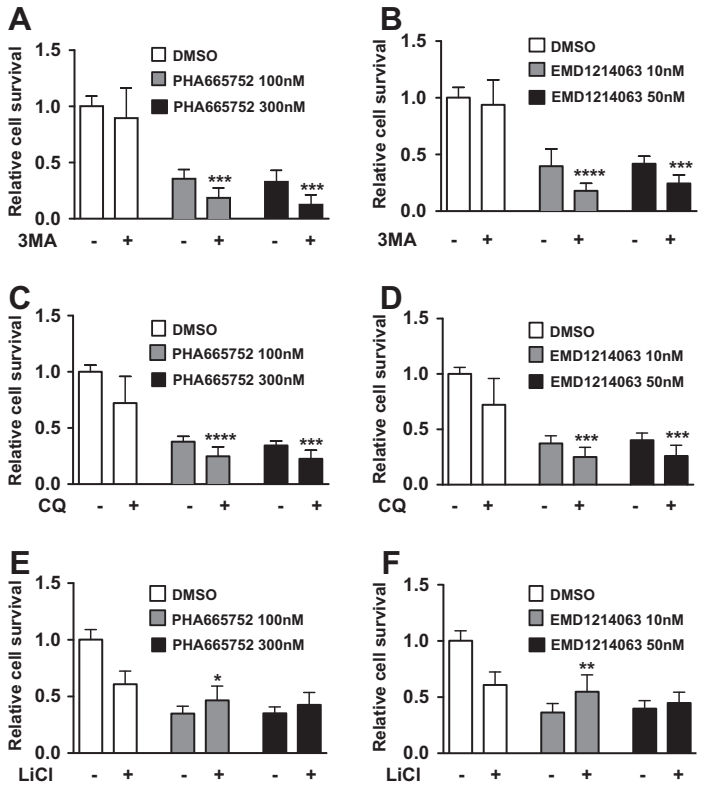

Fig. 2. Inhibiting autophagy in combination with MET inhibitors decreases cell viability. (A and B) GTL-16 cells were treated with PHA665752 (100 nM or 300 nM) or EMD1214063 (10 nM or 50 nM) with or without the autophagy inhibitor 3-MA (5 mM). Viability assays were performed at 24 h. Results were normalized to non-treated cells. (C and D) GTL-16 cells were treated with PHA665752 (100 nM or 300 nM) or EMD1214063 (10 nM or 50 nM) with or without CQ (25 μM). Assays were performed as in A. (E and F) GTL-16 cells were treated with PHA665752 (100 nM or 300 nM) or EMD1214063 (10 nM or 50 nM) with or without LiCl (25 mM). Assays were performed as in A.

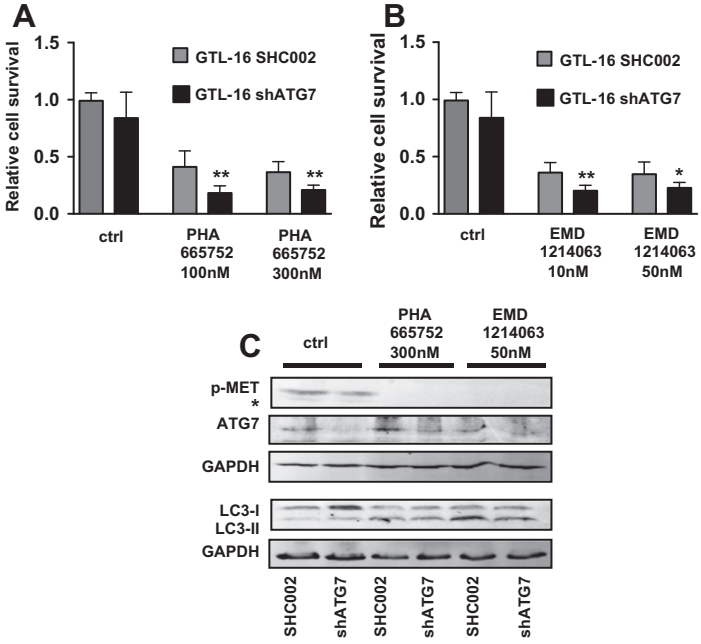

Fig. 3. Knocking down ATG7 decreases cell viability upon MET inhibitor treatment. (A and B) GTL-16 cells were transduced with SHC002 scramble control or shATG7 lentiviral vectors. SHC002 and shATG7 GTL16 cells were treated with PHA665752 or EMD1214063 at concentrations indicated. Viability assays were performed at 24 h. Results were normalized to non-treated cells. (C) Western blot analysis of phospho-MET, ATG7 and LC3-I LC3-II conversion. SHC002 and shATG7 GTL-16 cells were treated with EMD1214063 (50 nM) or PHA665752 (300 nM) for 24 h. Total protein was extracted from SHC002 or shATG7 GTL-16 cells and submitted to immunoblotting using anti-phospho-MET and anti-LC3B antibodies. GAPDH is shown as a loading control.

demonstrate that inhibiting MET in gastric carcinoma cells activates autophagy.

3.2. Modulating autophagy in combination with MET inhibition affects cell viability

To test if autophagy plays a role in protecting cells upon MET inhibition, we investigated cell viability upon treatment with MET inhibitors in combination with known modulators of autophagic activity. We performed viability assay on GTL-16 treated for 24 h with PHA665752 or EMD1214063 together with the autophagic inhibitors (3-MA or CQ) or activator (LiCl). Co-treatment with MET inhibitors and 3-MA, which inhibits early stages of autophagy, resulted in significantly reduced cell viability as compared to cells treated with MET inhibitors ($p < 0.05$) (Fig. 2A and B). In addition, co-treatment of GTL-16 cells with MET inhibitors and CQ, which impairs autophagy at late stage, showed similar effect (Fig. 2C and D). On the other hand, treatment with the autophagy activator LiCl repressed MET inhibitor-induced cell death (Fig. 2E and F). These results suggest that MET-induced autophagy represents a cytoprotective process and that a combination of MET- and autophagy inhibitors might be beneficial for future therapeutical approaches.

3.3. Genetic inhibition of autophagy decreases cell viability upon MET inhibition

To exclude non-autophagy effects of the autophagy inhibitors 3-MA and CQ, we genetically impaired autophagy. To this end we knocked down ATG7, a key player in autophagy using lentivirally delivered shRNA targeting ATG7. We treated GTL-16 SHC002 control and GTL-16 shATG7 autophagy-deficient cells with different concentration of MET inhibitors and measured cell viability. Similar to pharmacological inhibition of autophagy, knocking down ATG7 markedly decreased cell viability upon MET inhibitor treatment (Fig. 3A and B). ATG7 knockdown efficiency and decreased autophagic activity in ATG7 knockdown cells was confirmed by decreased LC3 I/LC3 II conversion (Fig. 3C). The efficiency of MET inhibitors was confirmed by p-MET Western blotting (Fig. 3C). Our autophagy knockdown data further support the hypothesis that autophagy is a prosurvival process during MET inhibition.

4. Discussion

In this study we found that the inhibition of the MET receptor in human gastric carcinoma cell lines GTL-16 and MKN45 induced autophagy. Pharmacological modulation of autophagy showed that MET inhibition-induced autophagy is cytoprotective in gastric carcinoma cells. To further support our data, we genetically blocked autophagy by generating ATG7 knockdown cells. We observed a decrease in cell viability in autophagy knockdown cells similar to cells were autophagy was blocked pharmacologically.

In general, MET deregulation in human cancer leads to several tumor-supportive mechanisms such as proliferative advantage, tumor cell dissemination, tumor invasion metastasis progression [1,23] as well as potentially providing resistance against DNA damaging modalities that are commonly used in cancer therapy [11,24]. Currently, MET is an attractive target in cancer therapy and, due to the potency of small molecule tyrosine kinase inhibitors in other receptor tyrosine kinase systems (e.g., Gleevec targeting the BCR–ABL fusion protein and the KIT receptor), small molecule inhibitors that would interfere with MET oncogenic activity represent a promising therapeutic strategy for many human cancers. However, since preclinical studies show that MET inhibition induces different cellular responses and resistance depending on the origin and type of tumor cells, it seems to be of a high importance to evaluate the impact of MET inhibition on particular cancer types [9].

In general, anti-cancer treatments induce stress in tumor cells, and these cells can respond by inducing autophagy allowing them to persist the chemotherapeutic insult. In such a case, inhibiting the autophagic stress response in parallel with conventional therapy would allow to inflict more cell death [20]. In contrast, autophagy could contribute to cell death upon treatment with genotoxic drugs. The majority of studies shows that autophagy is promoting cell survival upon therapy in many cellular systems [25–28]. Underlining the benefit of combining tyrosine kinase inhibitors with autophagy inhibitors stem from several studies. For example, combining the autophagy inhibitors 3-MA or CQ with the small molecule Src inhibitors (PP2 or saracatinib) increased the sensitivity of prostate cancer cells to the treatment [28]. In hepatocellular carcinoma cells, the anti-tumor potential of sorafenib, an inhibitor of several tyrosine protein kinase and Raf kinases inhibitor, is increased by co-treatment with the autophagy inhibitor chloroquine [29]. Moreover, Baf A or CQ enhance cell death in chronic myeloid leukemia (CML) cell lines and primary CML cells upon treatment with the abl kinase inhibitor imatinib [26]. We now add MET inhibition of gastric carcinoma cells to the list of therapies that could benefit from autophagy inhibition.

Anti-MET therapy combined with autophagy inhibitors has the potential to increase not only the cell toxicity but may also allow the use of lower concentration of MET inhibitors, hence decreasing the risk for unspecific toxicity in gastric cancer. Although *in vivo* data are needed to support our observations, our findings strongly suggest that autophagy is a cytoprotective process in gastric adenocarcinoma cells and that combining MET together with autophagy inhibitors has the potential to improve anti-MET therapies.

Authorship contributions

MH and MM performed the experimental research, interpreted the data and drafted the article. MFF and DMA instigated the experimental design and revised the drafted article. MPT prepared lentiviral vectors. MPT, FB, AB and YZ designed the project, analyzed data and gave final approval of the submitted manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.120.

References

[1] T. Nakamura, K. Sakai, T. Nakamura, K. Matsumoto, Hepatocyte growth factor twenty years on: much more than a growth factor, J. Gastroenterol. Hepatol. 26 (Suppl. 1) (2011) 188–202.

[2] K.A. Furge, Y.W. Zhang, G.F. Vande Woude, Met receptor tyrosine kinase: enhanced signaling through adapter proteins, Oncogene 19 (2000) 5582–5589.
[3] J. Yu, S. Miehlke, M.P. Ebert, J. Hoffmann, M. Breidert, B. Alpen, et al., Frequency of TPR-MET rearrangement in patients with gastric carcinoma and in first-degree relatives, Cancer 88 (2000) 1801–1806.
[4] I. Cañadas, F. Rojo, M. Arumí-Uría, A. Rovira, J. Albanell, E. Arriola, C-MET as a new therapeutic target for the development of novel anticancer drugs, Clin. Transl. Oncol. 12 (2010) 253–260.
[5] F. Cecchi, D.C. Rabe, D.P. Bottaro, Targeting the HGF/Met signalling pathway in cancer, Eur. J. Cancer 46 (2010) 1260–1270.
[6] S. Corso, C. Migliore, E. Ghiso, G. De Rosa, P.M. Comoglio, S. Giordano, Silencing the MET oncogene leads to regression of experimental tumors and metastases, Oncogene 27 (2007) 684–693.
[7] L. Trusolino, A. Bertotti, P.M. Comoglio, MET signalling: principles and functions in development, organ regeneration and cancer, Nat. Rev. Mol. Cell Biol. 11 (2010) 834–848.
[8] G.A. Smolen, R. Sordella, B. Muir, G. Mohapatra, A. Barmettler, H. Archibald, et al., Amplification of MET may identify a subset of cancers with extreme sensitivity to the selective tyrosine kinase inhibitor PHA-665752, Proc. Natl. Acad. Sci. USA 103 (2006) 2316–2321.
[9] J.G. Christensen, R. Schreck, J. Burrows, P. Kuruganti, E. Chan, P. Le, et al., A selective small molecule inhibitor of c-Met kinase inhibits c-Met-dependent phenotypes in vitro and exhibits cytoreductive antitumor activity in vivo, Cancer Res. 63 (2003) 7345–7355.
[10] S.S. Ganapathipillai, M. Medová, D.M. Aebersold, P.W. Manley, S. Berthou, B. Streit, et al., Coupling of mutated Met variants to DNA repair via Abl and Rad51, Cancer Res. 68 (2008) 5769–5777.
[11] M. Medová, D.M. Aebersold, W. Blank-Liss, B. Streit, M. Medo, S. Aebi, et al., MET inhibition results in DNA breaks and synergistically sensitizes tumor cells to DNA-damaging agents potentially by breaching a damage-induced checkpoint arrest, Genes Cancer 1 (2010) 1053–1062.
[12] J.P. Eder, G.F. Vande Woude, S.A. Boerner, P.M. LoRusso, Novel therapeutic inhibitors of the c-Met signaling pathway in cancer, Clin. Cancer Res. 15 (2009) 2207–2214.
[13] E. Jabbour, S.A. Parikh, H. Kantarjian, J. Cortes, Chronic myeloid leukemia: mechanisms of resistance and treatment, Hematol. Oncol. Clin. North Am. 25 (2011) 981–995.
[14] T. Kosaka, E. Yamaki, A. Mogi, H. Kuwano, Mechanisms of resistance to EGFR TKIs and development of a new generation of drugs in non-small-cell lung cancer, J. Biomed. Biotechnol. 2011 (2011) 165214.
[15] J.J. Lum, D.E. Bauer, M. Kong, M.H. Harris, C. Li, T. Lindsten, et al., Growth factor regulation of autophagy and cell survival in the absence of apoptosis, Cell 120 (2005) 237–248.
[16] A. Kuma, M. Hatano, M. Matsui, A. Yamamoto, H. Nakaya, T. Yoshimori, et al., The role of autophagy during the early neonatal starvation period, Nature 432 (2004) 1032–1036.
[17] M. Leist, M. Jäättelä, Four deaths and a funeral: from caspases to alternative mechanisms, Nat. Rev. Mol. Cell Biol. 2 (2001) 589–598.
[18] W. Bursch, A. Ellinger, H. Kienzl, L. Török, S. Pandey, M. Sikorska, et al., Active cell death induced by the anti-estrogens tamoxifen and ICI 164 384 in human mammary carcinoma cells (MCF-7) in culture: the role of autophagy, Carcinogenesis 17 (1996) 1595–1607.
[19] S. Chi, C. Kitanaka, K. Noguchi, T. Mochizuki, Y. Nagashima, M. Shirouzu, et al., Oncogenic Ras triggers cell suicide through the activation of a caspase-independent cell death program in human cancer cells, Oncogene 18 (1999) 2281–2290.
[20] E. White, R.S. DiPaola, The double-edged sword of autophagy modulation in cancer, Clin. Cancer Res. 15 (2009) 5308–5316.
[21] K. Degenhardt, R. Mathew, B. Beaudoin, K. Bray, D. Anderson, G. Chen, et al., Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis, Cancer Cell 10 (2006) 51–64.
[22] M. Rizzi, M.P. Tschan, C. Britschgi, A. Britschgi, B. Hügli, T.J. Grob, et al., The death-associated protein kinase 2 is up-regulated during normal myeloid differentiation and enhances neutrophil maturation in myeloid leukemic cells, J. Leukoc. Biol. 81 (2007) 1599–1608.
[23] G.M. Stella, S. Benvenuti, P.M. Comoglio, Targeting the MET oncogene in cancer and metastases, Expert. Opin. Invest. Drugs 19 (2010) 1381–1394.
[24] M. Medová, D.M. Aebersold, Y. Zimmer, MET inhibition in tumor cells by PHA665752 impairs homologous recombination repair of DNA double strand breaks, Int. J. Cancer 130 (2012) 728–734.
[25] W. Han, H. Pan, Y. Chen, J. Sun, Y. Wang, J. Li, et al., EGFR tyrosine kinase inhibitors activate autophagy as a cytoprotective response in human lung cancer cells, PLoS One 6 (2011) e18691.
[26] C. Bellodi, M.R. Lidonnici, A. Hamilton, G.V. Helgason, A.R. Soliera, M. Ronchetti, et al., Targeting autophagy potentiates tyrosine kinase inhibitor-induced cell death in Philadelphia chromosome-positive cells, including primary CML stem cells, J. Clin. Invest. 119 (2009) 1109–1123.
[27] A. Vazquez-Martin, C. Oliveras-Ferraros, J.A. Menendez, Autophagy facilitates the development of breast cancer resistance to the anti-HER2 monoclonal antibody trastuzumab, PLoS One 4 (2009) e6251.
[28] Z. Wu, P.-C. Chang, J.C. Yang, C.-Y. Chu, L.-Y. Wang, N.-T. Chen, et al., Autophagy blockade sensitizes prostate cancer cells towards Src family kinase inhibitors, Genes Cancer 1 (2010) 40–49.
[29] S. Shimizu, T. Takehara, H. Hikita, T. Kodama, H. Tsunematsu, T. Miyagi, et al., Inhibition of autophagy potentiates the antitumor effect of the multikinase inhibitor sorafenib in hepatocellular carcinoma, Int. J. Cancer 131 (2012) 548–557.