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RESEARCH

Heteronemin promotes iron-dependent cell death in pancreatic cancer

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Abstract
The marine environment has been recognized as a prolific source of potent bioactive compounds with significant anticancer properties. Among these, heteronemin, a sesterterpenoid-type natural product, has shown promise. This study delves into the potential of heteronemin as a ferroptotic agent against pancreatic cancer, using the Panc-1 cell line as a model. The cytotoxic potential of heteronemin was assessed using cell viability assays. Furthermore, its effect on lipid peroxidation was determined spectrophotometrically, while the changes it induced in autophagy- and ferritin-related protein expressions were evaluated using immunoblotting techniques. Various cell-based tests were employed to scrutinize its anticancer efficacy. Heteronemin displayed a notable cytotoxic effect, reducing cell viability by 50% at a concentration of 55 nM. This cytotoxicity was discernibly linked to ferroptosis, as evidenced by the reversal of cell death upon treatment with the ferroptosis inhibitor, ferrostatin-1. Heteronemin treatment led to a marked increase in ferroptosis markers and malondialdehyde (MDA) levels. Conversely, the expression of glutathione peroxidase-4 (GPX4), a key anti-ferroptotic protein, was suppressed. Furthermore, significant modulations in the expression of ferritinophagy- and iron-related proteins such as Atg5, Atg7, FTL, STEAP3, and DMT-1 were evident post-treatment ($p < 0.05$). This study underscores the potential of heteronemin as a ferroptosis inducer in pancreatic cancer cells. Given its robust cytotoxicity, heteronemin emerges as a promising lead compound for further exploration in cancer therapeutics.

Keywords Ferroptosis; Heteronemin · Pancreatic ductal adenocarcinoma · Sesterterpenoid-type natural product

Introduction

Pancreatic dysfunction manifests as a range of disorders from relatively benign conditions, such as diabetes and pancreatitis, to the more severe pancreatic cancers (Wolpin et al. 2009; Li et al. 2009; Blackford et al. 2009). Pancreatic ductal adenocarcinoma (PDAC), in particular, is notorious for its aggressive behavior and poor prognosis. Alarmingly, 80–90% of PDAC patients are diagnosed with local metastases, significantly limiting the possibility of surgical intervention (Grant et al. 2016; Fesinmeyer et al. 2005). Consequently, the predominant therapeutic modality for such cases remains chemotherapy, primarily involving agents like gemcitabine or a combination of 5-fluorouracil and leucovorin (Gill et al. 2016).

However, the innate resistance of PDAC cells to chemotherapy and their proclivity to evade apoptosis renders many current therapeutic strategies ineffective (Fitzgerald and McCubrey 2014; Ercan et al. 2017). Chemotherapy and/or radiotherapy often encounter limited success in the treatment of PDAC, a phenomenon partly attributed to the resistance of cancer cells to apoptosis. This resistance can be facilitated by a range of factors including alterations in apoptotic pathways and the influence of the tumor microenvironment, where components such as macrophages can play a pivotal role in promoting therapy resistance (DeNardo et al. 2009; Mitchem et al. 2013). This resistance often arises from genetic mutations and alterations in cellular pathways (Waddell et al. 2015; Siegel et al. 2020). Such factors, compounded by the early onset of metastasis, account for the dauntingly high mortality rate associated with PDAC. This emphasizes the dire need for new therapeutic modalities and the identification of early detection biomarkers (Vincent et al. 2011; Jones et al. 2008).

Ferroptosis is a form of cell death that is dependent on iron and reactive oxygen species, and it plays a role in several diseases including ischemic organ damage, neurodegeneration, and cancer (Dixon 2017; Yu et al. 2017;

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Xie et al. 2016; Mou et al. 2019; Li et al. 2019; Belaidi and Bush 2016; Ma et al. 2016; Ooko et al. 2015; Battaglia et al. 2020). Distinct from other forms of cell death like autophagy and apoptosis, ferroptosis is characterized by specific histological features such as heightened lipid peroxidation and reduced glutathione peroxidase-4 (GPX4) activity (Yang and Stockwell 2008). While unchecked ferroptosis can lead to various pathologies, including neurodegenerative disorders (Stockwell et al. 2017a), in the context of malignancy, it offers a tantalizingly novel approach to bypassing traditional apoptotic resistance (Hassannia et al. 2019).

Nature has historically been a generous provider of therapeutic agents. A vast array of current chemotherapeutics trace their origins to bioactive natural compounds, many of which have exhibited formidable anticancer activities (Newman and Cragg 2016; Ekor 2014; Nobili et al. 2009). Among these, marine-derived compounds, buoyed by the rich biodiversity of the oceans, offer a unique arsenal against cancers. Notable among these are compounds like pachymatismin, bryostatins, and heteronemin (Newman and Cragg 2014; Zidane et al. 1996; Khalifa et al. 2019). Heteronemin, specifically, a sesterterpenoid isolated from the sponge *Hyrtios* sp., has generated significant interest due to its potent cytotoxic effects across a spectrum of cancer cell types (Chang et al. 2021; Yang et al. 2021; Chen et al. 2018; Cheng et al. 2019; Lee et al. 2018; Saikia et al. 2018; Wu et al. 2015; Schumacher et al. 2010). Preliminary investigations, including our own, have hinted at heteronemin's potential to regulate ferroptosis in hepatocellular carcinoma (Chang et al. 2021). In a previous study conducted by Chang et al. (2021), the effects of heteronemin, a marine-derived terpenoid, on hepatocellular carcinoma (HCC) cell lines HA22T and HA59T were explored. The research demonstrated that heteronemin significantly inhibited the proliferation of these cell lines and induced apoptosis mediated through the caspase pathway. Moreover, heteronemin triggered the formation of reactive oxygen species (ROS), which were associated with cell death, and facilitated the removal of ROS through mitochondrial SOD2, rather than cytosolic SOD1. This induction of ROS was linked to the mitogen-activated protein kinase (MAPK) signaling pathway; heteronemin reduced the expression of ERK, a MAPK associated with cell proliferation, and the inhibitors of JNK and p38 MAPKs, which are associated with apoptosis, restored the cell death induced by heteronemin. Furthermore, the heteronemin treatment decreased the expression of GPX4, a protein that inhibits ferroptosis, a novel form of non-apoptotic cell death, and the treatment with a ferroptosis inhibitor also restored the cell death induced by heteronemin. These findings provide a mechanistic foundation that suggests heteronemin, with appropriate structural modifications, could function as a potential therapeutic agent against HCC (Chang et al. 2021). This naturally leads to an inquiry into its potential impact on pancreatic cancer cells.

Hence, this research aims to shed light on the efficacy and mechanistic action of heteronemin in orchestrating ferroptosis within the milieu of pancreatic cancer. A robust comprehension of this interplay could signify a monumental leap in our therapeutic approach to PDAC, potentially heralding hope for numerous afflicted individuals.

Materials and methods

Experimental design: cell culture and treatments

The human pancreatic ductal adenocarcinoma cell line (Panc-1, CRL-1469™, RRID: CVCL_0480) was bought from the American Type Culture Collection (ATCC, Manassas, VA, USA). The immortalized human keratinocyte cell line (HaCaT) was kindly gifted by Prof. Çiğdem Yenisey of Aydın Adnan Menderes University. Cells were maintained in DMEM containing 10% heat-inactivated fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured at 37 °C in a humidified incubator with 5% CO_2. For the experiments, the cells were treated with heteronemin at increasing concentrations (0.01–10 µM). The HaCaT cell line was used to study the potential cytotoxicity of heteronemin on normal cells. GPX4 antibody (sc-166570, RRID:AB_2112427) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Atg5 antibody (Cat# 12994, RRID:AB_2630393), Atg7 antibody (Cat# 8558, RRID:AB_10831194), anti-rabbit IgG, HRP-linked antibody (Cat# 7074, RRID:AB_2099233), and anti-mouse IgG, HRP-linked antibody (Cat# 7076, RRID:AB_330924) were purchased from Cell Signaling Biotechnology (Danvers, MA, USA). Ferritin light chain antibody (FNab03079), DMT1 (SLC11A2, FNab07905) and STEAP3 antibody (FNab08318) were purchased from Fine Test Wuhan Fine Biotech Corp. (Wuhan, China). All other compounds used in this study were purchased from Sigma-Aldrich (St. Louis, MO). Ethical approval is not applicable, because this article does not contain any studies with human or animal subjects.

Isolation of heteronemin from the sponge Hippospongia sp.

Heteronemin was separated from the marine sponge *Hippospongia* sp. following the same procedures in our previous report (Chen et al. 2018). In short, samples were collected from coral reefs off the coast of Taitung, Taiwan, by scuba diving at a depth of 20 m. Samples were freeze-dried and were extracted with EtOAc. Heteronemin was separated by a silica gel column with *n*-hexane–EtOAc (3:1) as the eluent solvent. The sample was further purified

on HPLC. An LC-20A VP HPLC system (Shimadzu Inc., Tokyo, Japan) was used for analysis equipped with a quaternary pump (LC-20AT), an online degasser (DGU-14A), a photodiode-array detector (SPD-M20A), an autosampler (SIL-20AD), and data collection using ClassVP. Cosmosil 5C-18-MS-II column (5 µm, 150 × 4.6 mm I.D.) supplied by Nacalai Tesque, Inc. (Kyoto, Japan) used for liquid chromatography. The samples were injected (10 µL), and the mobile phase consisted of water (A) and acetonitrile (B). A gradient program was applied as follows: the initial elution condition was A:B (25:75, v/v), linearly changed to A:B (12:88, v/v) at 10 min, A:B (4:96, v/v) at 15 min. The percentage of the mobile phase B increased linearly to 100% within 15 min and 210 nm was selected as the detection wavelength to collect the target compound.

Cell viability assay

Cellular viability was measured by an MTS assay kit (CellTiter 96 Aqueous One Solution, Promega). Cells were incubated in Hank's Balanced Salt Solution (1.3 mM $CaCl_2$, 0.5 mM $MgCl_2$, 0.4 mM $MgSO_4$, 5.3 mM KCl, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 137.9 mM NaCl, 0.3 mM Na_2HPO_4, 5.6 mM D-glucose) containing increasing concentrations of heteronemin (0.001–10 µM) and/or Fer-1 (0.5 µM) dissolved in DMSO. MTS reagent was added to each well following 48 h exposure. The absorbance was measured at 490 nm with a microplate spectrophotometer. The selective index was calculated by comparing the heteronemin IC_{50} value in the HaCaT cell line against the IC_{50} value in Panc-1.

Colony formation assay

Cells were seeded at 1×10^3 cells/well in 6-well plates and were treated with heteronemin and/or Fer-1. The culture media were replaced with new media twice a week for 2 weeks. Crystal violet (Sigma-Aldrich, St. Louis, MO) was used to stain and make colonies visible. Then, colonies were photographed and counted in three independent wells.

Lipid peroxidation assay

Lipid peroxidation product levels were evaluated by the method of Ohkawa et al. (1979). Briefly, the cells were incubated with heteronemin at an increasing concentration (0.01–1 µM) for 48 h. Then, the TBARS assay kit (Cayman Chemical) was used to measure malondialdehyde (MDA) levels in the sample at 532 nm.

Protein analysis

Cells (3×10^5/well) were treated with two different concentrations of heteronemin (1 and 10 µM) for 48 h. The cells were harvested and were lysed in a buffer containing a protease/phosphatase inhibitor cocktail. The cellular lysates were analyzed by Western blot analysis as previously described (Armagan et al. 2019).

Statistical analysis

The obtained data were expressed as mean ± standard deviation (SD) from three to five independent experiments performed in triplicate. The Shapiro–Wilk normality test was used to determine whether the data were normally distributed. The statistical comparisons were estimated using one-way ANOVA followed by the Tukey test using GraphPad Prism (GraphPad Software, Inc). p values lower than 0.05 were regarded as statistically significant.

Results

Heteronemin's cytotoxicity and selectivity in Panc-1 cells

Using an MTS cell viability assay kit, we assessed heteronemin's cytotoxic effects on both Panc-1 (a human pancreatic cancer cell line) and HaCaT (healthy immortalized human keratinocytes). After 48 h of treatment, heteronemin displayed a significantly lower IC50 value in Panc-1 cells (55 nM) compared to HaCaT cells (256 nM), indicating greater susceptibility of Panc-1 cells (Fig. 1). This distinction resulted in a selectivity index (SI) of 4.65 for heteronemin.

To further validate the induced ferroptotic pathway by heteronemin, we also employed erastin, a well-known ferroptosis inducer, in a parallel experiment as a positive control. Erastin's role in the study helped to benchmark the ferroptotic potential of heteronemin, providing a comprehensive understanding of heteronemin's capacity to induce ferroptosis in pancreatic cancer cells.

Furthermore, the application of the ferroptosis inhibitor, Fer-1, counteracted heteronemin-induced cellular death, suggesting ferroptosis as the underlying mechanism (Fig. 2).

Impact of heteronemin on Panc-1 colony formation

We observed a concentration-dependent decline in the colony-forming ability of Panc-1 cells with heteronemin treatment. Notably, the effect of heteronemin at a concentration of 1 µM was significantly reversed by the subsequent application of 0.5 µM Fer-1, restoring the colony-forming capability to 90%

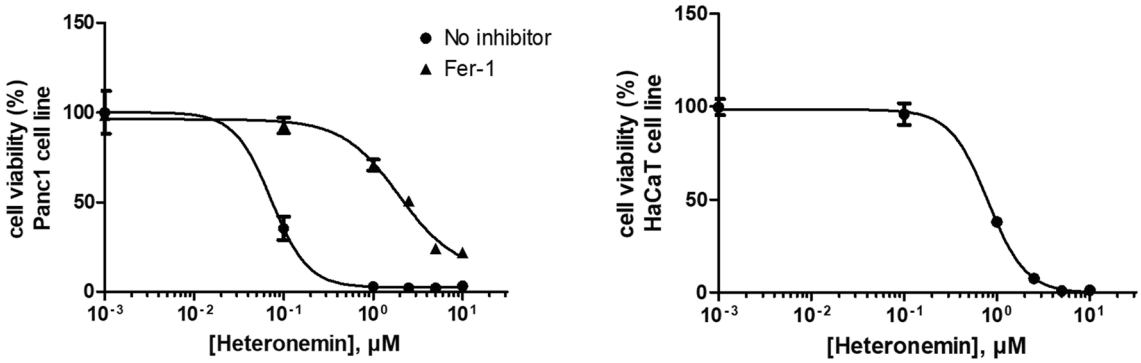

Fig. 1 Antiproliferative effect of heteronemin on Panc-1 and HaCaT cell lines at increasing concentrations (0.001, 0.1, 1, 2.5, 5, 10 µM). The results are expressed as percentage survival after 48 h of exposure. IC_{50} values were calculated as 0.055 µM (Panc-1) and 0.256 µM (HaCaT) for each cell line

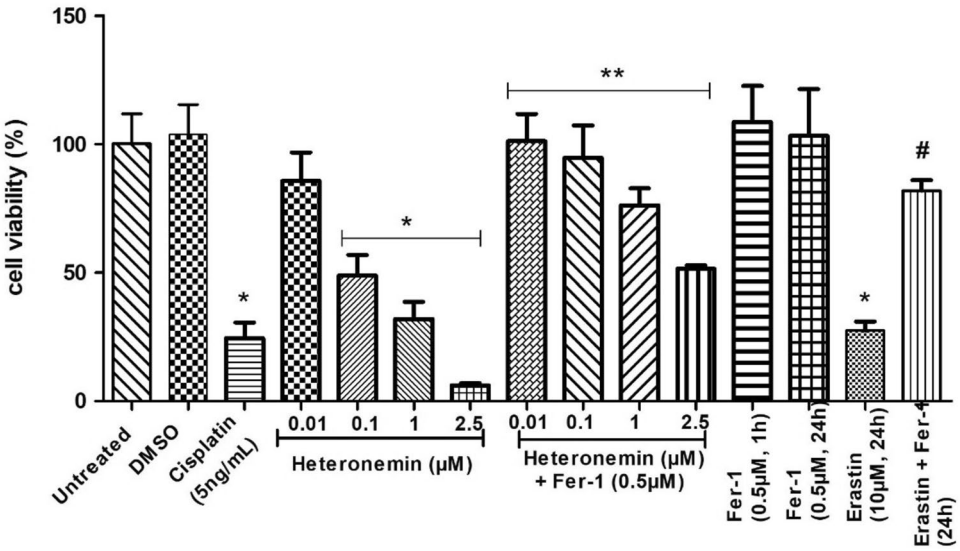

Fig. 2 Ferroptosis inducer potential of heteronemin on Panc-1 in the presence of ferroptosis inhibitor, Fer-1 (0.5 µM). The results are expressed as percentage survival after 48 h of exposure. $^{*}p < 0.05$ vs. untreated cells, $^{**}p < 0.05$ vs. heteronemin-only-treated cells at the same concentration. $^{\#}p < 0.05$ vs. erastin-treated cells

of the control group, thus underlining the pivotal role of ferroptosis in mediating the response to heteronemin (Fig. 3).

Lipid peroxidation and GPX4 expression under heteronemin treatment

High concentrations of heteronemin in Panc-1 cells led to a marked increase in the lipid peroxidation end-product, MDA, compared to both untreated and cisplatin-treated cells (Fig. 4). On the other hand, low concentrations did not induce any significant change in MDA levels. Aligning with this, heteronemin treatment at 1 µM significantly downregulated GPX4 protein expression ($p < 0.05$).

Modulation of ferritinophagy and iron-associated proteins by heteronemin

With the known role of autophagy in ferroptosis promotion through ferritin breakdown, we turned our focus to autophagy-related proteins: Atg5, Atg7, and the ferritin light chain subunit (FTL) (Hou et al. 2016). Heteronemin treatment resulted in the upregulation of Atg5 and Atg7 ($p < 0.05$). Contrary to our initial analysis, FTL levels did not exhibit a decrease at the highest concentration; rather, we observed a slight increase compared to the negative control (Fig. 5a). Furthermore, we observed a significant increase in protein expressions of divalent metal transporter-1 (DMT1) and six-transmembrane epithelial antigen of the prostate 3 (STEAP3), critical for iron transport from endosome to cytosol and reduction, after 48 h of heteronemin exposure ($p < 0.05$) (Fig. 5b). Collectively, these results emphasize heteronemin's potential to induce ferroptosis in Panc-1 cells by modulating iron transport and autophagy.

Discussion

Natural products extracted from distinct species significantly contributed to the development of effective therapeutics against all types of diseases. In this context, the ocean is of

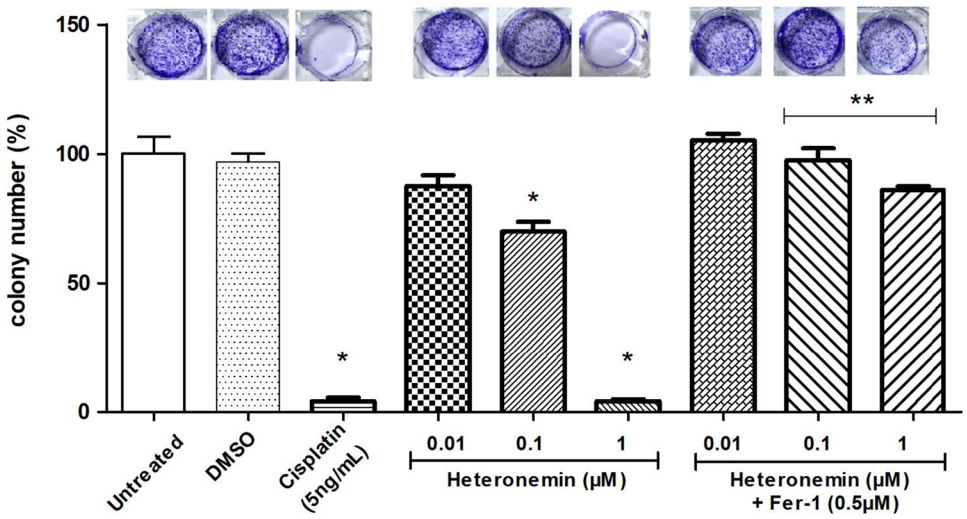

Fig. 3 Colony formation analysis of Panc-1 cells treated with cisplatin (5 ng/mL) heteronemin (0.01, 0.1, and 1 μM) and/or Fer-1 (0.5 μM) for 14 days. The bar graph represents the average of three biological replicates. Representative dishes stained with crystal violet. *$p < 0.05$ vs. untreated cells, **$p < 0.05$ vs. heteronemin-only-treated cells at the same concentration

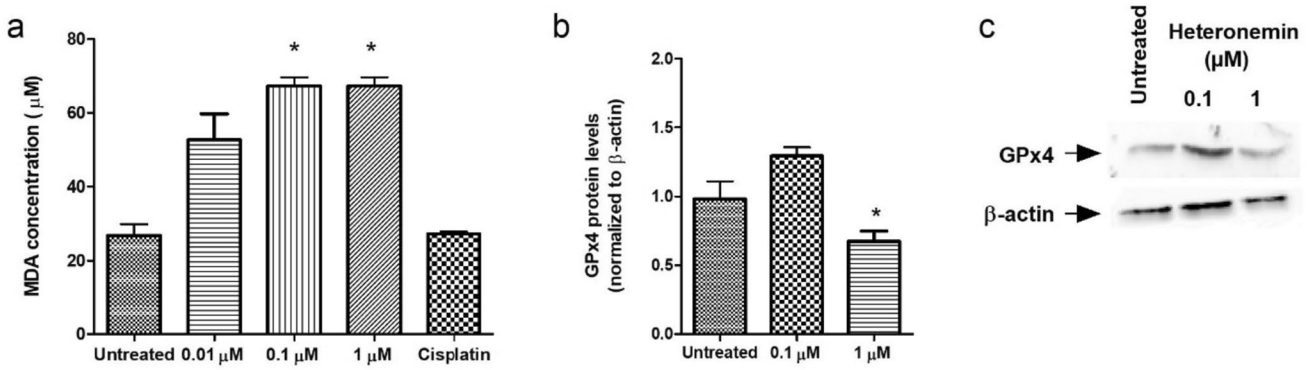

Fig. 4 Heteronemin induced lipid peroxidation and decreased GPx4 protein expression in Panc-1 cells. **a** MDA concentration was measured in cisplatin- and heteronemin-treated cells. **b**, **c** Bar graph data represent the mean ± SD; $n = 3$ independent experiments. Quantified band values of GPX4 were normalized to the corresponding β-actin signal. *$p < 0.05$ vs. untreated cells

immense importance as it has a large reservoir of marine species with their biologically active compounds possessing various activities including anticancer, anti-inflammatory, antimicrobial, and antioxidant (Wang et al. 2012; Villa and Gerwick 2010; Takamatsu et al. 2003; Gademann and Kobylinska 2009). Several marine-derived secondary metabolites such as alkaloids, terpenes, peptides, and steroids exhibit potent anticancer activities (Zhang et al. 2017; Kobayashı et al. 1994).

In the present study, we focused on heteronemin and evaluated its ferroptotic potential in a pancreatic cancer model. As previously reported, heteronemin exhibited anticancer, anti-nutritional, antimicrobial, protein inhibitory, and antitubercular activities (Gonzalez 2010; Wonganuchitmeta et al. 2004). In agreement with our findings, heteronemin reduced cell viability and proliferation in several cancer cell lines including leukemia, colon adenocarcinoma, breast cancer, and renal carcinoma at a concentration of less than one micromolar (Wu et al. 2020; Chang et al. 2012). In the present study, heteronemin showed potent cytotoxic activity against Panc-1 cells with IC_{50} of 55 nM. We observed a good selectivity profile with an SI value of 4.65 for heteronemin in pancreatic cancer cells following 48 h of treatment.

The most promising strategies for PDAC treatment are to inhibit mutated genes, such as KRAS, to regulate macromolecules that contribute to the disease progression, or to overcome chemoresistance (Adamska et al. 2018). The most used drugs approved by the FDA for pancreatic cancer are 5-fluorouracil, albumin-bound paclitaxel, cisplatin, gemcitabine, and FOLFIRINOX (5-fluorouracil, leucovorin, irinotecan, oxaliplatin) (Singh and O'Reilly 2020). These drugs have short half-lives and are usually administered in higher and repeated doses, which can induce a range of side effects from moderate to severe (Patra et al. 2010; Gyanani et al. 2021; Saad et al. 2004). Cisplatin is one of the agents used to treat pancreatic cancer. Severe side effects limit the

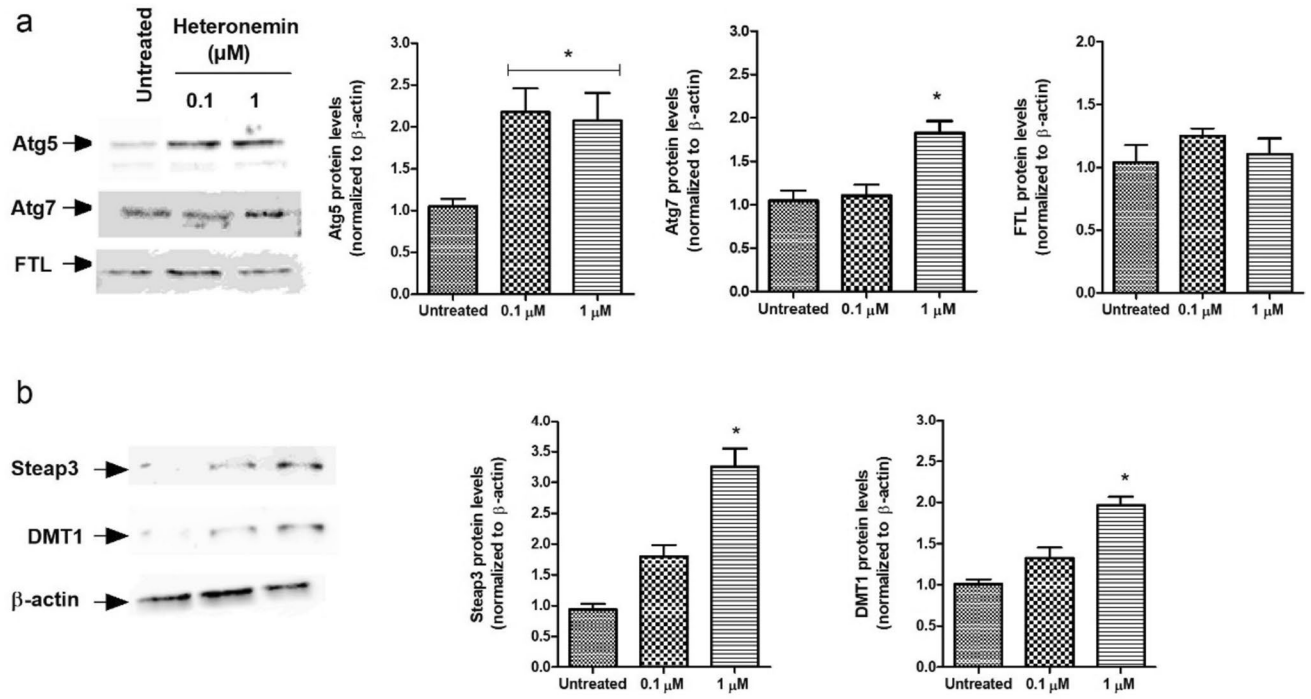

Fig. 5 Alteration in **a** ferritinophagy- (Atg5, Atg7, FTL) and **b** iron-related (STEAP3, DMT1) protein levels following heteronemin treatment in Panc-1. Quantified band values were normalized to the corresponding β-actin signal. Bar graph data represent the mean ± SD; $n = 3$ independent experiments. *$p < 0.05$ vs. untreated cells

therapeutic efficacy of cisplatin. Guo et al. (2018) reported that cisplatin inactivates GPX together with the induction of GSH depletion in cancer cells (Guo et al. 2018). Thus, we decided to use cisplatin as the positive control to compare its effect with heteronemin in PDAC. Similar to the inhibitory effect of heteronemin on cancer cell survival in the short-term culture, the long-term cell survival results monitored in a colony formation assay supported the inhibitory effect of heteronemin, which was comparable to cisplatin. Heteronemin-treated cells exhibited a reduced colony formation capacity as the concentration increased, and the results were comparable to cisplatin. These observations indicated that heteronemin selectively inhibited cell growth, and the results were comparable to the clinically used anticancer drugs in pancreatic cancer cells.

Chemotherapeutic agents disrupt cell homeostasis via inhibiting DNA synthesis, increasing oxidative stress, arresting the cell cycle, and inducing cellular death mechanisms such as necrosis and apoptosis. Although the Bcl-mediated apoptotic pathway and autophagy were reported to be induced by heteronemin in cancer cells (Lee et al. 2018; Wu et al. 2015), the effect of heteronemin on other cellular death pathways was not fully elucidated.

Recently, ferroptotic cell death is widely investigated in cancer studies (Ye et al. 2020; Lachaier et al. 2014). Most of the clinically used chemotherapeutic drugs were found to induce ferroptosis as well as apoptosis (Ye et al. 2020; Gao and Jiang 2018). In our previous study, we noted that the administration of the ferroptosis inhibitor Fer-1 successfully prevented cell death instigated by heteronemin. This finding aligns with our previous work demonstrating the capacity of a ferroptosis inhibitor to counteract heteronemin-induced cell death in hepatocellular carcinoma cells (Chang et al. 2021). Given these pivotal observations, we were encouraged to further delve into the analysis of ferroptosis induced by heteronemin in the current study. Based on the existing data, we formulated a hypothesis that heteronemin might be orchestrating the regulation of various pathways, including lipid peroxidation, iron transport, and iron storage, thus leading to the induction of ferroptosis in cancer cells.

Increasing evidence demonstrated those numerous metabolic pathways contribute to ferroptosis through lipid-ROS production (Stockwell et al. 2017b; Wu et al. 2020). Biochemical events including intracellular iron accumulation, and lipid peroxidation are critical for ferroptosis in cancer cells (Kang et al. 2019). Pathways inducing ferroptosis are associated with the reduction of cysteine uptake through the inhibition of system X_c^- (SLC7A11), the reduction of GPX4 activity, and eventually the accumulation of intracellular lipid peroxides (Tarangelo et al. 2018; Anderson and Frazer 2017). GPX4 serves as a pivotal enzyme in suppressing the onset of ferroptosis, primarily through its role in detoxifying lipid peroxides. Previously, the ability of heteronemin to reduce GPX4 protein expression was reported

in hepatocellular carcinoma cell lines (Chang et al. 2021). In our study, GPX4 protein expression was significantly decreased in response to heteronemin as well. The downregulation of GPX4 by heteronemin together with the increased MDA levels indicated that heteronemin successfully inhibited the lipid peroxidation product scavenging activity of GPX4 and promoted ferroptosis in pancreatic cancer cells.

One of the components that distinguish ferroptosis from other cell death mechanisms is iron metabolism. Free Fe^{2+} causes ferroptosis by catalyzing free radical formation via the Fenton reaction. Biochemically, the reduction of Fe^{3+} to Fe^{2+} is catalyzed by STEAP3 in the endosome. Fe^{2+} is released into the cytoplasm via DMT1 (Tang et al. 2018). Thus, any alteration in the expression of these proteins is critical for the labile iron pool and the consequent maintenance of iron homeostasis. Turcu et al. (2020) reported that blockade of DMT1 inhibits iron translocation which leads to lysosomal iron overload and ferroptosis in cancer stem cells (Turcu et al. 2020). However, the upregulation of STEAP3 and DMT1 in pancreatic cancer cells following heteronemin treatment indicated that the conversion of Fe^{3+} to Fe^{2+} as well as the release of free Fe^{2+} into cytoplasm may be triggered by heteronemin. Ferritinophagy is defined as the degradation of ferritin, providing free Fe^{2+} for the cell, and contributing to ferroptosis as a source of unstable iron ions (Yu et al. 2017; Tang et al. 2018). Previously, Atg5 and Atg7 knockdown/knockout were demonstrated to block erastin-induced ferroptosis with decreased intracellular ferrous iron levels and lipid peroxidation (Hou et al. 2016) Conversely, upregulation of Atg5 and Atg7 protein expressions in response to heteronemin treatment, accompanied by a slight increase in ferritin light chain (FTL) protein level, could potentially facilitate a nuanced mechanism involved in the induction of ferroptosis in cancer cells. Despite the central role that decreased FTL typically plays in facilitating ferroptosis through enhancing iron availability, its slight increase in our study poses an interesting avenue of exploration.

It is plausible that the slight increase in FTL is indicative of a compensatory mechanism being activated in the cells to moderate the ferroptotic process initiated by the elevated levels of iron-incorporating proteins, representing a homeostatic effort to retain iron within a bound state and limit its availability for participation in reactions generating lipid peroxides. Moreover, FTL can have various roles in different cellular contexts and its function might be influenced by other intracellular dynamics, including its interaction with other molecules and pathways, potentially playing a role in the regulation of cellular responses to heteronemin treatment.

Indeed, the maintenance of a certain level of FTL might be crucial to prevent unrestrained ferroptosis and ensure cell survival under conditions of moderate stress, thus showcasing a delicate balance in the regulation of ferroptotic pathways. These observations open up a field of inquiry into the multi-faceted roles that FTL might play in the context of ferroptosis, including potential protective mechanisms that might be activated in response to ferroptotic agents.

Furthermore, it would be pertinent to investigate whether the slight increase in FTL levels is associated with alterations in the functioning of other components of the iron metabolism pathway, potentially revealing a more complex and nuanced network of interactions and regulatory mechanisms. Uncovering the detailed mechanisms and the reasons behind the unaltered FTL levels could, therefore, add a new dimension to our understanding of the heteronemin-induced ferroptotic pathway in Panc-1 cells.

As our findings present a slight increase in FTL levels, which diverge from the expected decrease usually observed in ferroptotic pathways, future studies should aim to unravel the underlying mechanisms through detailed investigations, possibly illuminating novel regulatory aspects of ferroptosis and opening avenues for more targeted therapeutic strategies in pancreatic cancer treatment.

A study conducted in 2008 found that cancer cells undergoing ferroptosis increased iron import and decreased iron storage compared to other cells (Yang and Stockwell 2008). Thus, it can be suggested that heteronemin sensitizes tumor cells to ferroptosis by modulating iron metabolism. Reduced iron storage because of decreased FTL and increased autophagy-related protein expression in response to heteronemin may contribute to iron overload and eventually trigger ferroptosis in cancer cells.

Conclusion

Pancreatic cancers are resistant to the currently used drugs. To overcome drug-resistance mechanisms such as increased drug efflux, improved DNA repair, and impaired apoptosis, activating ferroptotic pathway is a state-of-the-art therapeutic strategy. Taken together, our present study demonstrated that heteronemin promoted ferroptosis in pancreatic cancer cells via the regulation of several proteins that possess critical roles in the progression of ferroptosis. We believe that our study will be of importance to understanding the heteronemin mechanism of action as a potential anticancer drug. Heteronemin itself, or its derivatives to be synthesized in the future with higher selectivity and affinity, will be highly promising agents for patients suffering from pancreatic cancer.

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Author contributions All authors contributed to the study conception and design. Gizem Kaftan, Mümin Alper Erdoğan and Güliz Armagan performed cell culture studies and prepared figures. Mei-Chin Lu and Hung-Yu Lin isolated heteronemin. The first draft of the manuscript was written by Güliz Armagan and Mohamed El-Shazly. Luciano Saso and Shou-Ping Shih revised first draft. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Availability of data and materials The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval Not applicable.

Competing interests The authors declare no competing interests.

References

Adamska A, Elaskalani O, Emmanouilidi A, Kim M, Razak NBA, Metharom P, Falasca M (2018) Molecular and cellular mechanisms of chemoresistance in pancreatic cancer. Adv Biol Regul 68:77–87. https://doi.org/10.1016/j.jbior.2017.11.007

Anderson GJ, Frazer DM (2017) Current understanding of iron homeostasis. Am J Clin Nutr 106:1559S-1566S. https://doi.org/10.3945/ajcn.117.155804

Armagan G, Sevgili E, Gürkan FT, Köse FA, Bilgiç T, Dagcı T, Saso L (2019) Regulation of the Nrf2 pathway by glycogen synthase kinase-3β in MPP^+-induced cell damage. Molecules 24:1377. https://doi.org/10.3390/molecules24071377

Battaglia AM, Chirillo R, Aversa I, Sacco A, Costanzo F, Biamonte F (2020) Ferroptosis and cancer: mitochondria meet the "iron maiden" cell death. Cells 9:1505. https://doi.org/10.3390/cells9061505

Belaidi AA, Bush AI (2016) Iron neurochemistry in Alzheimer's disease and Parkinson's disease: targets for therapeutics. J Neurochem 139:179–197. https://doi.org/10.1111/jnc.13425

Blackford A, Parmigiani G, Kensler TW, Wolfgang C, Jones S, Zhang X, Parsons DW, Lin JCH, Leary RJ, Eshleman JR, Goggins M, Jaffee EM, Iacobuzio-Donahue CA, Maitra A, Klein A, Cameron JL, Olino K, Schulick R, Winter J, Vogelstein B, Velculescu VE, Kinzler KW, Hruban RH (2009) Genetic mutations associated with cigarette smoking in pancreatic cancer. Cancer Res 69:3681–4388. https://doi.org/10.1158/0008-5472.CAN-09-0015

Chang YC, Tseng SW, Liu LL, Chou Y, Ho YS, Lu MC, Su JH (2012) Cytotoxic sesterterpenoids from a sponge Hippospongia sp. Mar Drugs 10:987–997. https://doi.org/10.3390/md10050987

Chang WT, Bow YD, Fu PJ, Li CY, Wu CY, Chang YH, Teng YN, Li RN, Lu MC, Liu YC, Chiu CC (2021) A marine terpenoid, heteronemin, induces both the apoptosis and ferroptosis of hepatocellular carcinoma cells and involves the ROS and MAPK pathways. Oxid Med Cell Longev 7689045. https://doi.org/10.1155/2021/7689045

Chen YC, Lu MC, El-Shazly M, Lai KH, Wu TY, Hsu YM, Lee YL, Liu YC (2018) Breaking down leukemia walls: Heteronemin, a sesterterpene derivative, induces apoptosis in leukemia Molt4 cells through oxidative stress, mitochondrial dysfunction and induction of talin expression. Mar Drugs 16(6):212. https://doi.org/10.3390/md16060212

Cheng MH, Huang HL, Lin YY, Tsui KH, Chen PC, Cheng SY, Chong IW, Sung PJ, Tai MH, Wen ZH, Chen NF, Kuo HM (2019) BA6 induces apoptosis via stimulation of reactive oxygen species and inhibition of oxidative phosphorylation in human lung cancer cells. Oxid Med Cell Longev. https://doi.org/10.1155/2019/6342104

DeNardo DG, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, Coussens LM (2009) CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. Cancer Cell 16(2):91–102. https://doi.org/10.1016/j.ccr.2009.06.018

Dixon SJ (2017) Ferroptosis: bug or feature? Immunol Rev 277:150–157. https://doi.org/10.1111/imr.12533

Ekor M (2014) The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 4:177. https://doi.org/10.3389/fphar.2013.00177

Ercan G, Karlitepe A, Ozpolat B (2017) Pancreatic cancer stem cells and therapeutic approaches. Anticancer Res 7:2761–2775. https://doi.org/10.21873/anticanres.11628

Fesinmeyer MD, Austin MA, Li CI, De Roos AJ, Bowen DJ (2005) Differences in survival by histologic type of pancreatic cancer. Cancer Epidemiol Biomark Prev 14:1766–1773. https://doi.org/10.1158/1055-9965.EPI-05-0120

Fitzgerald TL, McCubrey JA (2014) Pancreatic cancer stem cells: association with cell surface markers, prognosis, resistance, metastasis and treatment. Adv Biol Regul 56:45–50. https://doi.org/10.1016/j.jbior.2014.05.001

Gademann K, Kobylinska J (2009) Antimalarial natural products of marine and freshwater origin. Chem Rec 9:187–198. https://doi.org/10.1002/tcr.200900001

Gao M, Jiang X (2018) To eat or not to eat—the metabolic flavor of ferroptosis. Curr Opin 51:58–64. https://doi.org/10.1016/j.ceb.2017.11.001

Gill S et al (2016) PANCREOX: a randomized phase III study of fluorouracil/leucovorin with or without oxaliplatin for second-line advanced pancreatic cancer in patients who have received gemcitabine-based chemotherapy. J Clin Oncol 34(32):3914–3920. https://doi.org/10.1200/JCO.2016.68.5776

Gonzalez MA (2010) Scalarane sesterterpenoids. Curr Bioact Compd 6:178–206. https://doi.org/10.2174/157340710793237362

Grant TJ, Hua K, Singh A (2016) Molecular pathogenesis of pancreatic cancer. Prog Mol Biol Transl Sci 44:241–275. https://doi.org/10.1016/bs.pmbts.2016.09.008

Guo J, Xu B, Han Q, Zhou H, Xia Y, Gong C, Dai X, Li ZGW (2018) Ferroptosis: a novel anti-tumor action for cisplatin. Cancer Res Treat 50:445. https://doi.org/10.4143/crt.2016.572

Gyanani V, Haley JC, Goswami R (2021) Challenges of current anticancer treatment approaches with focus on liposomal drug delivery systems. Pharmaceuticals (Basel) 14(9):835. https://doi.org/10.3390/ph14090835

Hassannia B, Vandenabeele P, Vanden Berghe T (2019) Targeting ferroptosis to iron out cancer. Cancer Cell 35(6):830–849

Hou W, Xie Y, Song X, Sun X, Lotze MT, Zeh HJ III, Kang R, Tang D (2016) Autophagy promotes ferroptosis by degradation of ferritin. Autophagy 12:1425–1428. https://doi.org/10.1080/15548627.2016.1187366

Jones S, Zhang X, Parsons DW, Lin JCH, Leary RJ, Angenendt P ... Carter H (2008) Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 321(5897):1801–1806

Kang R, Kroemer G, Tang D (2019) The tumor suppressor protein p53 and the ferroptosis network. Free Radic Biol Med 133:162–168. https://doi.org/10.1016/j.freeradbiomed.2018.05.074

Khalifa SA, Elias N, Farag MA, Chen L, Saeed A, Hegazy MEF, Moustafa MS, El-Wahed AA, Al-Mousawi SM, Musharraf SG, Chang FR, Iwasaki A, Kiyotake Suenaga K, Alajlani M, Göransson U, El-Seedi HR (2019) Marine natural products: a source of novel anticancer drugs. Mar Drugs 17:491. https://doi.org/10.3390/md17090491

Kobayashı M, Okamoto T, Hayashı K, Yokoyama N, Sasakı T (1994) Kitagawa I Marine natural products XXXII Absolute configurations of C-4 of the manoalide family, biologically active sesterterpenes from the marine sponge Hyrtions erecta. Chem Pharm Bull 42:265–270. https://doi.org/10.1248/cpb.42.265

Lachaier E, Louandre C, Godin C, Saidak Z, Baert M, Diouf M, Chauffert B, Galmiche A (2014) Sorafenib induces ferroptosis in human cancer cell lines originating from different solid tumors. Anticancer Res 34:6417–6422

Lee MG, Liu YC, Lee YL, El-Shazly M, Lai KH, Shih SP, Ke SC, Hong MC, Du YC, Yang JC, Sung PJ, Wen ZH, Lu MC (2018) Heteronemin, a marine sesterterpenoid-type metabolite, induces apoptosis in prostate LNcap cells via oxidative and ER stress combined with the inhibition of topoisomerase II and Hsp90. Mar Drugs 16:204. https://doi.org/10.3390/md16060204

Li D, Yeung SC, Hassan MM, Konopleva M, Abbruzzese JL (2009) Antidiabetic therapies affect risk of pancreatic cancer. Gastroenterology 137:482–488. https://doi.org/10.1053/j.gastro.2009.04.013

Li Y, Feng D, Wang Z, Zhao Y, Sun R, Tian D, Liu D, Zhang F, Ning S, Yao J, Tian X (2019) Ischemia-induced ACSL4 activation contributes to ferroptosis-mediated tissue injury in intestinal ischemia/reperfusion. Cell Death Differ 26:2284–2299. https://doi.org/10.1038/s41418-019-0299-4

Ma S, Henson ES, Chen Y, Gibson SB (2016) Ferroptosis is induced following siramesine and lapatinib treatment of breast cancer cells. Cell Death Dis 7:e2307–e2307. https://doi.org/10.1038/cddis.2016.208

Mitchem JB, Brennan DJ, Knolhoff BL, Belt BA, Zhu Y, Sanford DE ... Linehan DC (2013) Targeting tumor-infiltrating macrophages decreases tumor-initiating cells, relieves immunosuppression, and improves chemotherapeutic responses. Cancer Res 73(3):1128–1141. https://doi.org/10.1158/0008-5472.CAN-12-2731

Mou Y, Wang J, Wu J, He D, Zhang C, Duan C, Li B (2019) Ferroptosis, a new form of cell death: opportunities and challenges in cancer. J Hematol Oncol 12:1–16. https://doi.org/10.1186/s13045-019-0720-y

Newman DJ, Cragg GM (2014) Marine-sourced anti-cancer and cancer pain control agents in clinical and late preclinical development. Mar Drugs 12:255–278. https://doi.org/10.3390/md12010255

Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79:629–661. https://doi.org/10.1021/acs.jnatprod.5b01055

Nobili S, Lippi D, Witort E, Donnini M, Bausi L, Mini E, Capaccioli S (2009) Natural compounds for cancer treatment and prevention. Pharmacol Res 59:365–378. https://doi.org/10.1016/j.phrs.2009.01.017

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358. https://doi.org/10.1016/0003-2697(79)90738-3

Ooko E, Saeed ME, Kadioglu O, Sarvi S, Colak M, Elmasaoudi K, Janah R, Greten HR, Efferth T (2015) Artemisinin derivatives induce iron-dependent cell death (ferroptosis) in tumor cells. Phytomedicine 22:1045–1054. https://doi.org/10.1016/j.phymed.2015.08.002

Patra CR, Bhattacharya R, Mukhopadhyay D, Mukherjee P (2010) Fabrication of gold nanoparticles for targeted therapy in pancreatic cancer. Adv Drug Deliv Rev 62:346–361. https://doi.org/10.1016/j.addr.2009.11.007

Saad SY, Najjar TA, Alashari M (2004) Role of non-selective adenosine receptor blockade and phosphodiesterase inhibition in cisplatin-induced nephrogonadal toxicity in rats. Clin Exp Pharmacol Physiol 31:862–867. https://doi.org/10.1111/j.1440-1681.2004.04127.x

Saikia M, Retnakumari AP, Anwar S, Anto NP, Mittal R, Shah S, Pillai KS, Balachandran VS, Peter V, Thomas R, Anto RJ (2018) Heteronemin, a marine natural product, sensitizes acute myeloid leukemia cells towards cytarabine chemotherapy by regulating farnesylation of Ras. Oncotarget 9:18115. https://doi.org/10.18632/oncotarget.24771

Schumacher M, Cerella C, Eifes S, Chateauvieux S, Morceau F, Jaspars M, Dicato M, Diederich M (2010) Heteronemin, a spongean sesterterpene, inhibits TNFα-induced NF-κB activation through proteasome inhibition and induces apoptotic cell death. Biochem Pharmacol 79:610–622. https://doi.org/10.1016/j.bcp.2009.09.027

Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020. CA: Cancer J Clinic 70(1):7–30

Singh RR, O'Reilly EM (2020) New treatment strategies for metastatic pancreatic ductal adenocarcinoma. Drugs 80:647–669. https://doi.org/10.1007/s40265-020-01304-0

Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ ... Jiang X (2017a) Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell 171(2):273–285

Stockwell BR, Angeli JPF, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE, Noel K, Jiang X, Linkermann A, Murphy ME, Overholtzer M, Oyagi A, Pagnussat GC, Park J, Ran Q, Rosenfeld CS, Salnikow K, Tang D, Torti FM, Torti SV, Toyokuni S, Woerpel KA, Zhang DD (2017b) Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell 171:273–285. https://doi.org/10.1016/j.cell.2017.09.021

Takamatsu S, Hodges TW, Rajbhandari I, Gerwick WH, Hamann MT, Nagle DG (2003) Marine natural products as novel antioxidant prototypes. J Nat Prod 66:605–608. https://doi.org/10.1021/np0204038

Tang M, Chen Z, Wu D, Chen L (2018) Ferritinophagy/ferroptosis: iron-related newcomers in human diseases. J Cell Physiol 233:9179–9190. https://doi.org/10.1002/jcp.26954

Tarangelo A, Magtanong L, Bieging-Rolett KT, Li Y, Ye J, Attardi LD, Dixon SJ (2018) p53 suppresses metabolic stress-induced ferroptosis in cancer cells. Cell Rep 22:569–575. https://doi.org/10.1016/j.celrep.2017.12.077

Turcu AL, Versini A, Khene N, Gaillet C, Cañeque T, Müller S, Rodriguez R (2020) DMT1 inhibitors kill cancer stem cells by blocking lysosomal iron translocation. Chem Eur J 26:7369–7373. https://doi.org/10.1002/chem.202000159

Villa FA, Gerwick L (2010) Marine natural product drug discovery: leads for treatment of inflammation, cancer, infections, and neurological disorders. Immunopharmacol Immunotoxicol 32:228–237. https://doi.org/10.3109/08923970903296136

Vincent A, Herman J, Schulick R, Hruban RH, Goggins M (2011) Pancreatic cancer. Lancet 378(9791):607–620

Waddell N, Pajic M, Patch AM, Chang DK, Kassahn KS, Bailey P ... Johns AL (2015) Whole genomes redefine the mutational landscape of pancreatic cancer. Nature 518(7540), 495–501

Wang W, Wang SX, Guan HS (2012) The antiviral activities and mechanisms of marine polysaccharides: an overview. Mar Drugs 10:2795–2816. https://doi.org/10.3390/md10122795

Wolpin BM, Chan AT, Hartge P, Chanock SJ, Kraft P, Hunter DJ, Giovannucci EL, Fuchs CS (2009) ABO blood group and the risk of pancreatic cancer. J Natl Cancer Inst 101:424–431. https://doi.org/10.1093/jnci/djp020

Wonganuchitmeta SN, Yuenyongsawad S, Keawpradub N, Plubrukarn A (2004) Antitubercular sesterterpenes from the Thai sponge Brachiaster sp. J Nat Prod 67:1767–1770. https://doi.org/10.1021/np0498354

Wu SY, Sung PJ, Chang YL, Pan SL, Teng CM (2015) Heteronemin, a spongean sesterterpene, induces cell apoptosis and autophagy in

human renal carcinoma cells. Biomed Res Int 738241, 13 pages. https://doi.org/10.1155/2015/738241
Wu Y, Zhang S, Gong X, Tam S, Xiao D, Liu S, Tao Y (2020) The epigenetic regulators and metabolic changes in ferroptosis-associated cancer progression. Mol Cancer 19:1–17. https://doi.org/10.1186/s12943-020-01157-x
Xie Y, Hou W, Song X, Yu Y, Huang J, Sun X, Kang R, Tang D (2016) Ferroptosis: process and function. Cell Death Differ 23:369–379. https://doi.org/10.1038/cdd.2015.158
Yang WS, Stockwell BR (2008) Synthetic lethal screening identifies compounds activating iron-dependent, nonapoptotic cell death in oncogenic-RAS-harboring cancer cells. Chem Biol 15:234–245. https://doi.org/10.1016/j.chembiol.2008.02.010
Yang YCS, Li ZL, Huang TY, Su KW, Lin CY, Huang CH, Chen HY, Lu MC, Huang HW, Lee SY, Whang-Peng J, Lin HY, Davis PJ, Wang K (2021) Effect of estrogen on heteronemin-induced antiproliferative effect in breast cancer cells with different estrogen receptor status. Front Cell Dev Biol 2000. https://doi.org/10.3389/fcell.2021.688607
Ye Z, Liu W, Zhuo Q, Hu Q, Liu M, Sun Q, Zhang Z, Fan G, Xu W, Ji S, Yu X, Qin Y, Xu X (2020) Ferroptosis: Final destination for cancer? Cell Prolif 53:e12761. https://doi.org/10.1111/cpr.12761
Yu H, Guo P, Xie X, Wang Y, Chen G (2017) Ferroptosis, a new form of cell death, and its relationships with tumourous diseases. J Cell Mol Med 21:648–657. https://doi.org/10.1111/jcmm.13008
Zhang H, Zhao Z, Wang H (2017) Cytotoxic natural products from marine sponge-derived microorganisms. Mar Drugs 15:68. https://doi.org/10.3390/md15030068
Zidane M, Pondaven P, Roussakis C, More MT (1996) Effects in vitro of pachymatismin, a glycoprotein from the marine sponge Pachymatisma johnstonii, on a non-small-cell bronchopulmonary carcinoma line (NSCLC-N6). Anticancer Res 5A:2805–2812

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