

# S-Adenosyl-L-methionine-competitive inhibitors of the histone methyltransferase EZH2 induce autophagy and enhance drug sensitivity in cancer cells

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The enhancer of zeste homolog 2 (EZH2) has emerged as a novel anticancer target. Various EZH2 inhibitors have been developed in recent years. Among these, 3-deazaneplanocin A (DZNep) is known to deplete EZH2 protein expression through an indirect pathway. In contrast, GSK343 directly inhibits enzyme activity through an S-adenosyl-L-methionine-competitive pathway. Therefore, we proposed that DZNep and GSK343 may exert differential effects against cancer cells. In this study, we found that GSK343 but not DZNep induced autophagic cell death of cancer cells. Inhibition of EZH2 expression was not required for GSK343-induced autophagy. In addition, GSK343 enhanced the anticancer activity of a multikinase inhibitor, sorafenib, in human hepatocellular carcinoma cells. Our results show that GSK343 is a more potent anticancer agent than DZNep, and for the first time, we show that it acts as an autophagy

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## Introduction

Polycomb group gene (PcG) proteins, which act as transcriptional repressors silencing specific sets of genes through chromatin modification, are key players in various epigenetic phenomena. PcG proteins are grouped into two categories on the basis of functional association with distinct classes of multimeric complexes, termed polycomb repressive complexes (PRC1 and PRC2). PRC2, which contains the H3K27-specific histone methyltransferase enhancer of zeste homolog 2 (EZH2) and other PcG proteins (SUZ12 and EED), is responsible for monomethylation, dimethylation, and trimethylation at histone H3K27 (H3K27-me1/2/3). Overexpression of EZH2 has been found in tumors and inhibits the expression of tumor suppressor genes [1–3]. EZH2 can recruit histone deacetylase through EED, and cooperatively represses transcription [1,3,4]. Therefore, inhibition of EZH2 could reactivate tumor-suppressive genes and might be a potential anticancer treatment [5,6].

Over the past few years, several potent inhibitors of EZH2 have been discovered [7]. Among these, 3-deazaneplanocin A (DZNep), an S-adenosyl-L-homocysteine (SAH) hydrolase inhibitor, acts as an indirect inhibitor by

depleting EZH2 and the associated H3K27-me3 [6]. DZNep induces apoptosis of cancer cells, which is partially related to its ability to inhibit the PRC2 pathway, although the exact mechanism has not yet been elucidated [6]. Another major class of EZH2 inhibitors includes S-adenosyl-L-methionine (SAM)-competitive inhibitors. SAM is a universal methyl donor for catalytic reactions of histone methyltransferases. Several SAM-competitive inhibitors, such as EPZ005687, EI1, GSK126, and GSK343, have been discovered, which can selectively kill lymphoma cells harboring EZH2-activating mutations [8–11].

Autophagy is a physiological process involved in the turnover of proteins and intracellular organelles [12]. Cellular events during autophagy follow three distinct stages: initiation (formation of the phagophore), elongation (growth and closure of the autophagosome), and maturation (fusion of a double-membrane autophagosome into an autolysosome) [13,14]. Cytosolic proteins and organelles are sequestered into autophagosomes, and then degraded by lysosomal hydrolases after fusion with lysosomes. A group of autophagy-related proteins (Atg proteins) were isolated and characterized [15]. Among these, two ubiquitin-like conjugation systems, Atg12 and LC3 (also known as Atg8), are essential for the formation of autophagosomes. LC3 exists in either cytosolic LC3-I (autophagy-inactive) or processed LC3-II (autophagy-active) forms. LC3-II is localized in autophagosome

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membranes and the level of LC3-II expression is proportional to that of autophagic vacuoles. LC3-II trapped in inner membranes of autophagosomes is ultimately degraded during their maturation into autolysosomes [16]. Autophagy serves as a temporary survival mechanism during starvation; however, long-term deprivation of nutrients ultimately leads to excess self-digestion and autophagic cell death [12].

In this study, we found that DZNep and GSK343 exerted differential effects on cancer cells. GSK343 but not DZNep induced autophagic cell death. Inhibition of EZH2 was not required for GSK343-induced autophagy. GSK343 also enhanced the anticancer activity of sorafenib. Our results indicate that DZNep and GSK343 may exert anticancer effects through different pathways. In addition, we show for the first time that SAM-competitive EZH2 inhibitors (including GSK343 and UNC1999) induce autophagy in cancer cells.

## Materials and methods

### Materials

LC3B, p62, LAMP2, trimethylated histone H3K27, GAPDH, and  $\beta$ -actin antibodies were purchased from GeneTex (Hsinchu, Taiwan). The PARP1 antibody was purchased from Cell Signaling Technology (Beverly, Massachusetts, USA). The caspase-3 antibody was purchased from Imgenex (San Diego, California, USA). Dulbecco's modified Eagle medium, fetal bovine serum (FBS), L-glutamine, penicillin, and streptomycin sulfate were obtained from Gibco (Gaithersburg, Maryland, USA). GSK343 and UNC1999 were kindly provided by the Structural Genomics Consortium at the University of Toronto (Toronto, Ontario, Canada). DZNep and 3-methyladenine (3-MA) were purchased from Cayman Chemical (Ann Arbor, Michigan, USA). Bafilomycin A1 and sorafenib were purchased from LC Laboratories (Woburn, Massachusetts, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), berberine, doxorubicin, taxol, and VP-16 were purchased from Sigma Chemical (St Louis, Missouri, USA). siGENOME human *EZH2* SMARTpool siRNA, siGENOME non-targeting siRNA pool, and DharmaFECT 4 siRNA transfection reagent were purchased from Dharmacon (Lafayette, Colorado, USA).

### Cell culture

MDA-MB-231 human breast cancer, A549 human lung cancer, and HepG2 human liver cancer cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% FBS, 0.03% (w/v) L-glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate. Cultures were maintained at 37°C and 5% CO<sub>2</sub> in a humidified incubator.

### Cell viability assay

Cell viability was measured using an MTT assay. Cells were plated in 96-well plates and treated with drugs. After 72 h of incubation, 0.5 mg/ml of MTT was added to each well for an additional 4 h. The blue MTT formazan precipitate was then dissolved in 200  $\mu$ l of dimethyl sulfoxide. The absorbance at 550 nm was measured on a multiwell plate reader.

### Western blot analysis

Cells were lysed in an ice-cold buffer containing 50 mmol/l Tris-HCl (pH 7.5), 150 mmol/l NaCl, 1 mmol/l MgCl<sub>2</sub>, 2 mmol/l EDTA, 1% NP-40, 10% glycerol, 1 mmol/l DTT, 1 $\times$  protease inhibitor cocktail, and 1 $\times$  phosphatase inhibitor cocktail at 4°C for 30 min. Cell lysates (25–50  $\mu$ g) were separated on a 7–12% SDS-polyacrylamide gel and then transferred electrophoretically onto a nitrocellulose membrane. The membrane was prehybridized in 20 mmol/l Tris-HCl (pH 7.5), 150 mmol/l NaCl, 0.05% Tween-20 (TBST buffer), and 5% skim milk for 1 h, and then transferred to a solution containing 1% BSA/TBST and a primary antibody and incubated overnight at 4°C. After washing with the TBST buffer, the membrane was submerged in 1% BSA/TBST containing a horseradish peroxidase-conjugated secondary antibody for 1 h. The membrane was washed with TBST buffer and then developed using an enhanced chemiluminescence system (Perkin-Elmer, Boston, Massachusetts, USA) and exposed to a radiographic film.

### Fluorescence microscopic analysis of autophagic vacuoles

The formation of autophagic vacuoles was monitored using a Cyto-ID autophagy detection kit (Enzo Life Sciences, Farmingdale, New York, USA) following the manufacturer's protocol. Briefly, cells were washed twice in PBS containing 5% FBS and then stained with Cyto-ID detection reagent and Hoechst 33342 (Enzo Life Sciences). After 30 min of incubation at 37°C, cells were washed and examined by fluorescence microscopy.

### siRNA knockdown analyses

Human *EZH2* and control small interfering RNAs (siRNAs) were transiently transfected into cells with DharmaFECT 4 siRNA transfection reagent according to the manufacturer's instructions. Briefly, 50% confluent cells in 6-cm dishes were transfected with 100 pmol siRNA and 10  $\mu$ l of transfection reagent in 4 ml of antibiotics-free complete medium for 24 h at 37°C. Then, the transfection mixture was replaced with fresh complete medium and cells were cultured for an additional 48 h. Then, cells were lysed and protein expression was analyzed by western blot analysis.

### Statistical analysis

Means and SDs of samples were calculated from the numerical data generated in this study. Data were analyzed using Student's *t*-test, and *P* values less than 0.05 were considered significant.

## Results

### Differential effects of DZNep and GSK343 on the cell viability and autophagy of cancer cells

Inhibition of EZH2 has recently been considered an attractive therapeutic approach for the treatment of cancer. DZNep is the first discovered small molecule that indirectly depletes EZH2 protein expression and inhibits H3K27-me3 and H4K20-me3 [6,17]. DZNep acts as an inhibitor of SAH hydrolase. SAH is the byproduct of EZH2-mediated methylation. Elevation of SAH by DZNep in turn serves as a byproduct inhibitor of methylation reactions (Fig. 1a) [18]. In contrast, GSK343 was developed as a direct and selective EZH2 inhibitor through competitively binding to the methyl donor, SAM [11]. Therefore, we propose that GSK343 may be a more potent anticancer agent than DZNep. Indeed, although treatment with 5  $\mu$ mol/l DZNep reduced the cell viability of human breast cancer MDA-MB-231 cells to 67%, higher doses did not further reduce cell viability (Fig. 1b). Unlike DZNep, GSK343 showed cytotoxicity toward MDA-MB-231 cells in a dose-dependent manner (Fig. 1b). However, western blot analysis showed that both DZNep and GSK343 reduced the level of H3K27-me3 in MDA-MB-231 cells (Fig. 1c), suggesting that the differential effects of DZNep and GSK343 might not result from their abilities to inhibit EZH2.

To investigate whether DZNep and GSK343 induce apoptosis, PARP and caspase-3 expressions were examined by western blot analysis. A DNA damage agent, doxorubicin, was used as a positive control and this drug induced apoptosis as indicated by the cleavage of PARP and caspase-3 (Fig. 1d). However, both GSK343 and DZNep did not induce the cleavage of caspase-3 and only a residual amount of cleaved PARP was detected (Fig. 1d). These results suggest that induction of apoptosis is not responsible for the inhibition of cell viability by DZNep and GSK343, although GSK343 was found to reduce the level of the proform of caspase-3 (Fig. 1d). Autophagy and apoptosis may be triggered in independent (parallel) or mutually exclusive manners, and these two phenomena in combination decide a cell's fate [19]. The ability of DZNep and GSK343 to induce autophagy was evaluated by LC3-II accumulation. Only GSK343 could induce LC3-II accumulation (Fig. 1d). These results indicate that DZNep and GSK343 exert different effects on cell viability and autophagy of MDA-MB-231 cells.

### GSK343 but not DZNep is a potent autophagy inducer in cancer cells

To further characterize the differential effects of DZNep and GSK343 on autophagy, MDA-MB-231 cells were

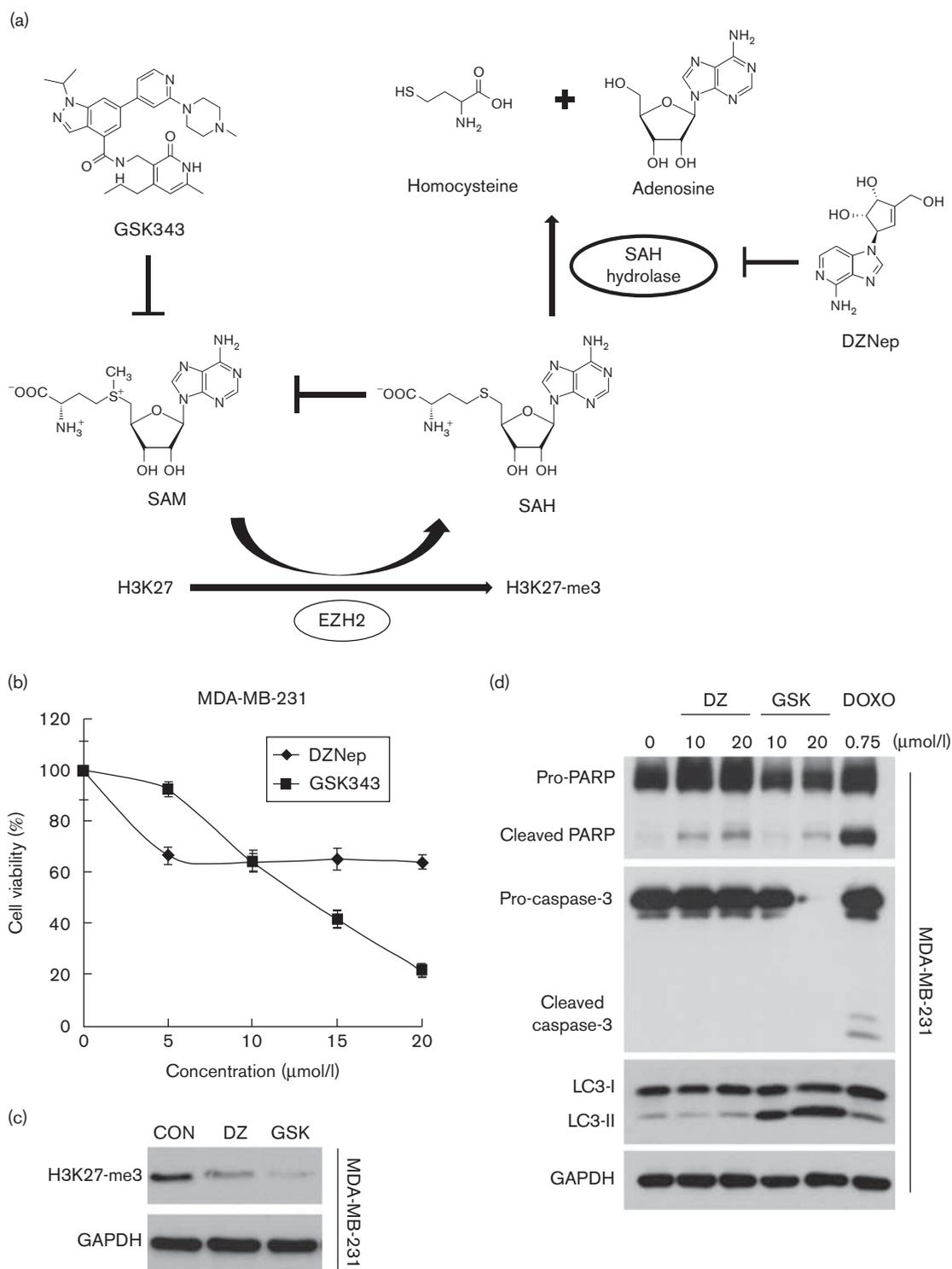
treated with DZNep and GSK343 for 24 h and autophagy was evaluated by LC3-II accumulation. Our results show that GSK343 but not DZNep induced LC3-II accumulation (Fig. 2a). Because LC3-II can accumulate through impairment of downstream autophagosome–lysosome fusion, LC3 flux was examined in the presence of bafilomycin A1, a vacuolar-type H<sup>+</sup>-ATPase inhibitor that blocks autophagosome–lysosome fusion. As shown in Fig. 2b, GSK343 still enhanced LC3-II accumulation in the presence of bafilomycin A1. SQSTM1/p62 is degraded by autophagy [20]. Although both DZNep and GSK343 reduced p62 expression, only GSK343-induced p62 downregulation could be blocked by bafilomycin A1 (Fig. 2b). In addition, the total amount of LAMP2, a lysosomal membrane-associated protein [21], was not altered after DZNep or GSK343 treatment with or without bafilomycin A1 (Fig. 2b). These results suggest that GSK343-induced LC3-II accumulation was not because of inhibition of autophagic degradation. The formation of autophagic vacuoles was monitored by Cyto-ID fluorescence. Similarly, only GSK343 increased the Cyto-ID fluorescence intensity (Fig. 2c). Furthermore, GSK343's effect of inducing autophagy was compared with other anticancer reagents. As shown in Fig. 2d, the efficacy of GSK343 in inducing LC3-II accumulation in MDA-MB-231 cells was higher than those of the other drugs. To investigate whether other SAM-competitive EZH2 inhibitors could induce autophagy, another novel EZH2 inhibitor, UNC1999 (Fig. 2e) [22], was used to treat MDA-MB-231 cells. Indeed, UNC1999 also induced LC3-II accumulation (Fig. 2d). These results indicate that SAM-competitive EZH2 inhibitors are potent autophagy inducers in MDA-MB-231 cells.

To investigate whether the differential effects of DZNep and GSK343 on cell viability and autophagy were ubiquitous in cancer cells, human liver cancer HepG2 and lung cancer A549 cells were treated with various doses of DZNep and GSK343 and the reduction of H3K27-me3 by these drugs was first confirmed by western blot analysis (Fig. 3a). The effects of DZNep and GSK343 on the cell viability of HepG2 and A549 cells were further analyzed using an MTT assay. As observed in MDA-MB-231 cells, differential effects of DZNep and GSK343 on cell viability were also found in HepG2 and A549 cells (Fig. 3b). In addition, GSK343 but not DZNep induced LC3-II accumulation in the absence and presence of bafilomycin A1 (Fig. 3c and d). Therefore, GSK343 acts as a general autophagy inducer in cancer cells.

### Knockdown of EZH2 expression is insufficient to induce autophagy

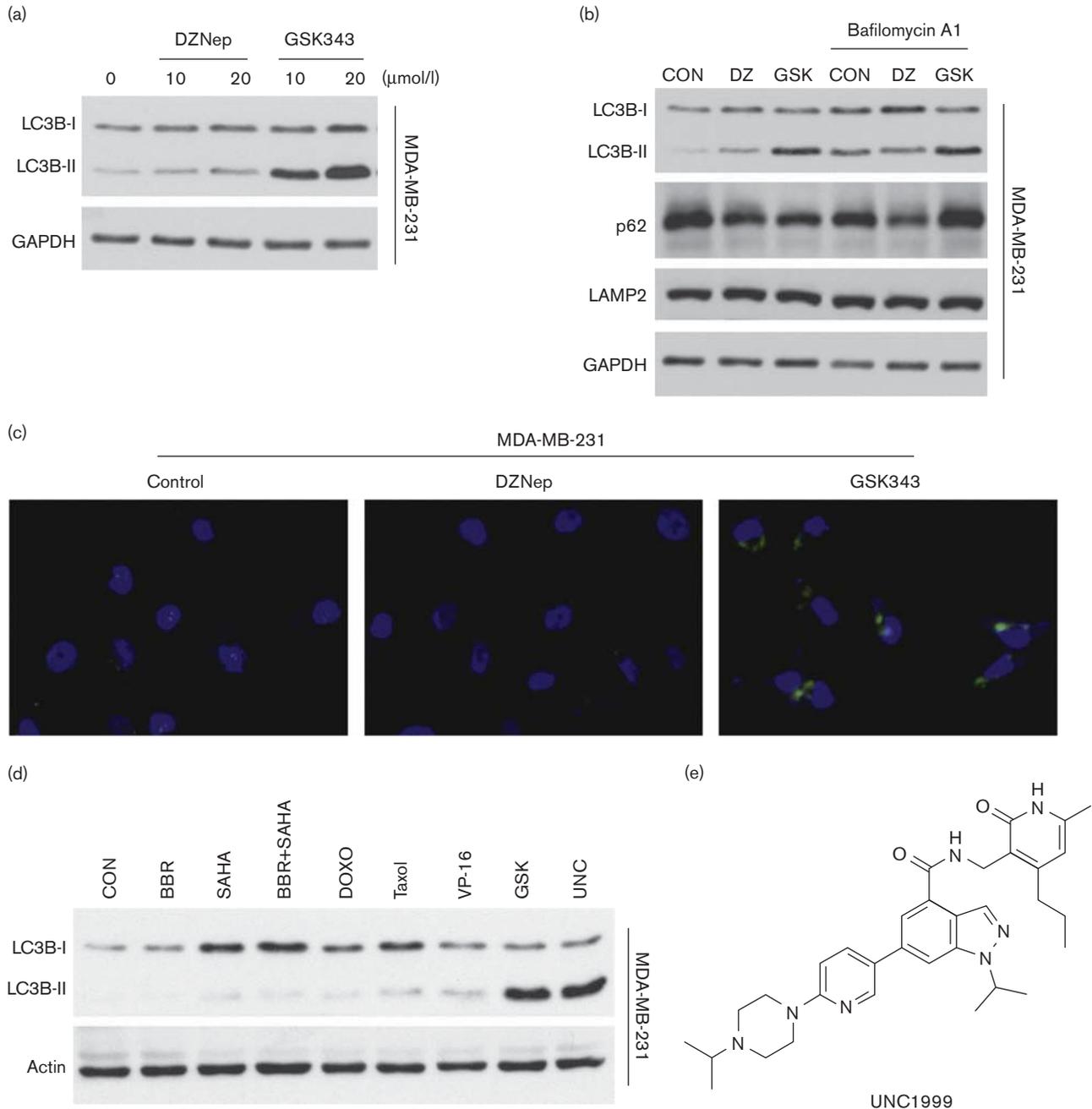
These differential effects of DZNep and GSK343 imply that inhibition of EZH2 is not required for inducing autophagy. To confirm the role of EZH2 in autophagy, MDA-MB-231, HepG2, and A549 cells were transfected with *EZH2* siRNA, and protein expression was analyzed

Fig. 1



Effects of DZNep and GSK343 on the cell viability of MDA-MB-231 cells. (a) Chemical structures of DZNep and GSK343. (b) MDA-MB-231 cells were treated with different doses of DZNep or GSK343 for 72 h, and cell viability was analyzed using an MTT assay. (c) MDA-MB-231 cells were treated with 20 μmol/l DZNep or 10 μmol/l GSK343 for 72 h, and whole-cell lysates were subjected to a western blot analysis using antibodies against H3K27-me3 or GAPDH. (d) MDA-MB-231 cells were treated with 10 and 20 μmol/l DZNep or GSK343, or 0.75 μmol/l doxorubicin (DOXO) for 72 h, and whole-cell lysates were subjected to a western blot analysis using antibodies against PARP1, caspase-3, LC3B, or GAPDH. DZNep, 3-deazaneplanocin A; EZH2, enhancer of zeste homolog 2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; SAH, S-adenosyl-L-homocysteine; SAM, S-adenosyl-L-methionine.

Fig. 2

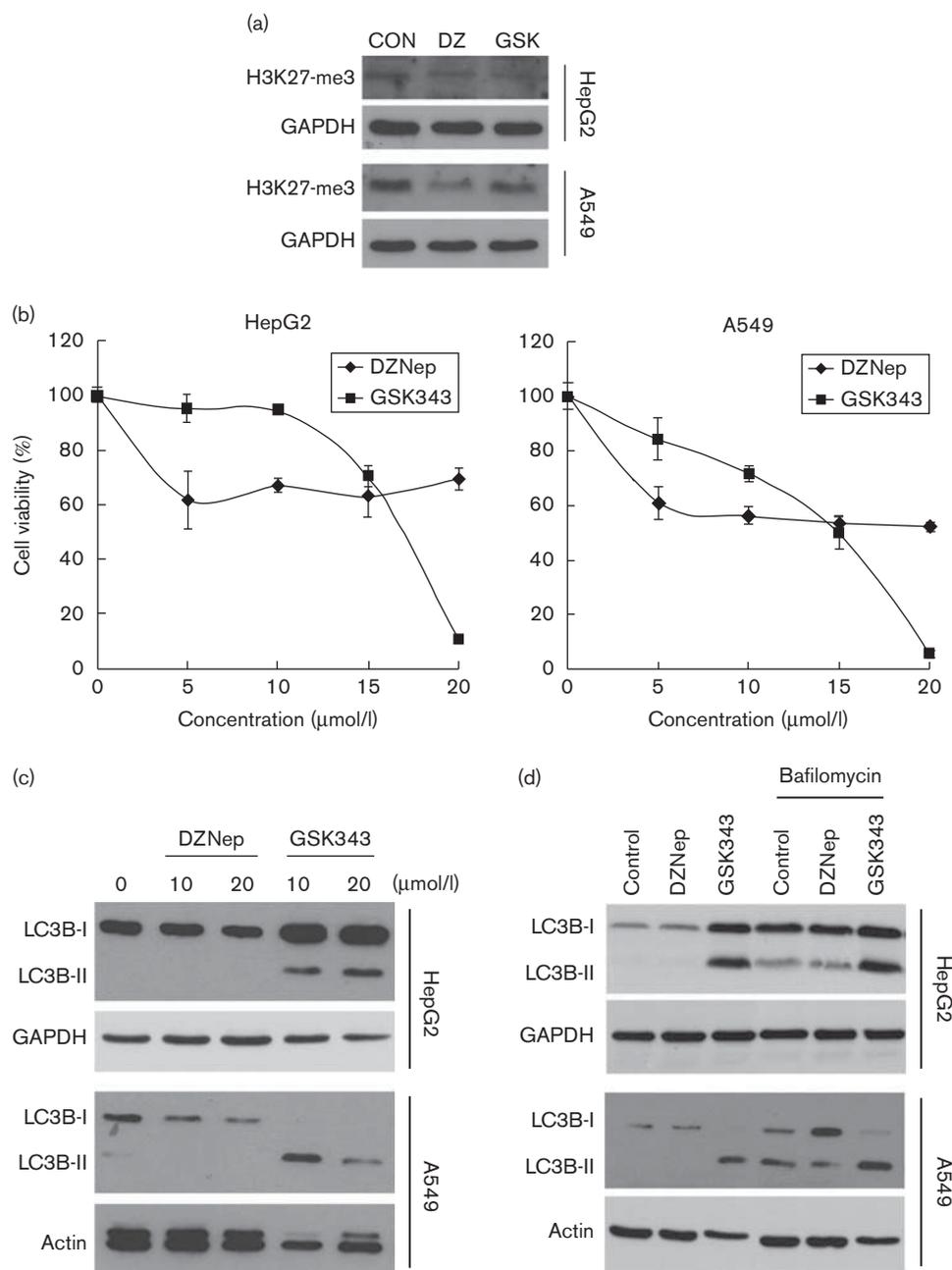


Effects of DZNep and GSK343 on autophagy of MDA-MB-231 cells. (a) MDA-MB-231 cells were treated with 10 and 20  $\mu\text{mol/l}$  DZNep or GSK343 for 24 h, and whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B or GAPDH. (b) MDA-MB-231 cells were treated with 20  $\mu\text{mol/l}$  DZNep or 10  $\mu\text{mol/l}$  GSK343 for 24 h, and 20 nmol/l bafilomycin was added 4 h before cells were harvested. Whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B, p62, LAMP2, or GAPDH. (c) MDA-MB-231 cells were treated with 20  $\mu\text{mol/l}$  DZNep or 10  $\mu\text{mol/l}$  GSK343 for 24 h, and then stained by Cyto-ID autophagy detection reagent. The Cyto-ID fluorescence was analyzed by fluorescence microscopy. (d) MDA-MB-231 cells were treated with 10  $\mu\text{mol/l}$  berberine (BBR), 2  $\mu\text{mol/l}$  SAHA, 1  $\mu\text{mol/l}$  doxorubicin (DOXO), 1  $\mu\text{mol/l}$  taxol, 10  $\mu\text{mol/l}$  VP-16, 10  $\mu\text{mol/l}$  GSK343 (GSK), or 10  $\mu\text{mol/l}$  UNC1999 (UNC) for 48 h, and whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B or  $\beta$ -actin. (e) The chemical structure of UNC1999, 3-deazaneplanocin A.

by western blot analysis. As shown in Fig. 4, knockdown of EZH2 expression did not induce LC3-II accumulation, suggesting that depletion of EZH2 is insufficient to

induce autophagy. Interestingly, the level of p62 was reduced by *EZH2* siRNA (Fig. 4), which was similar to the effect of DZNep (Fig. 2b).

Fig. 3



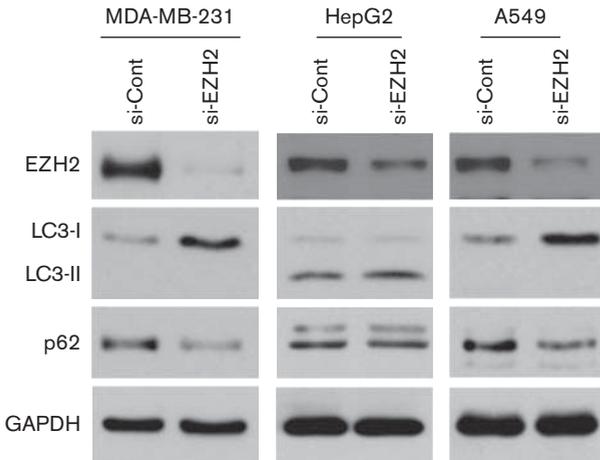
Effects of DZNep and GSK343 on the cell viability and autophagy of HepG2 and A549 cells. (a) HepG2 and A549 cells were treated with 20  $\mu\text{mol/l}$  DZNep or 10  $\mu\text{mol/l}$  GSK343 for 72 h, and whole-cell lysates were subjected to a western blot analysis using antibodies against H3K27-me3 or GAPDH. (b) HepG2 and A549 cells were treated with different doses of DZNep or GSK343 for 72 h, and the cell viability was analyzed using an MTT assay. (c) HepG2 and A549 cells were treated with 10 and 20  $\mu\text{mol/l}$  DZNep or GSK343 for 24 h, and whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B, GAPDH, or  $\beta$ -actin. (d) HepG2 and A549 cells were treated with 20  $\mu\text{mol/l}$  DZNep or 10  $\mu\text{mol/l}$  GSK343 for 24 h, and 20 nmol/l bafilomycin was added 4 h before cells were harvested. Whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B, GAPDH, or  $\beta$ -actin. DZNep, 3-deazaneplanocin A; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

### GSK343 induces autophagic cell death in cancer cells

The basic cellular functions of autophagy in eukaryotic cells are required to achieve a balance between protein synthesis and organelle biogenesis versus protein degradation and organelle turnover; thus, autophagy acts as a

cytoprotective mechanism under normal circumstances [19]. However, in several scenarios, autophagy constitutes a stress adaptation method that avoids cell death, whereas in other situations, autophagy represents an alternative pathway to cellular death [19]. To investigate

Fig. 4



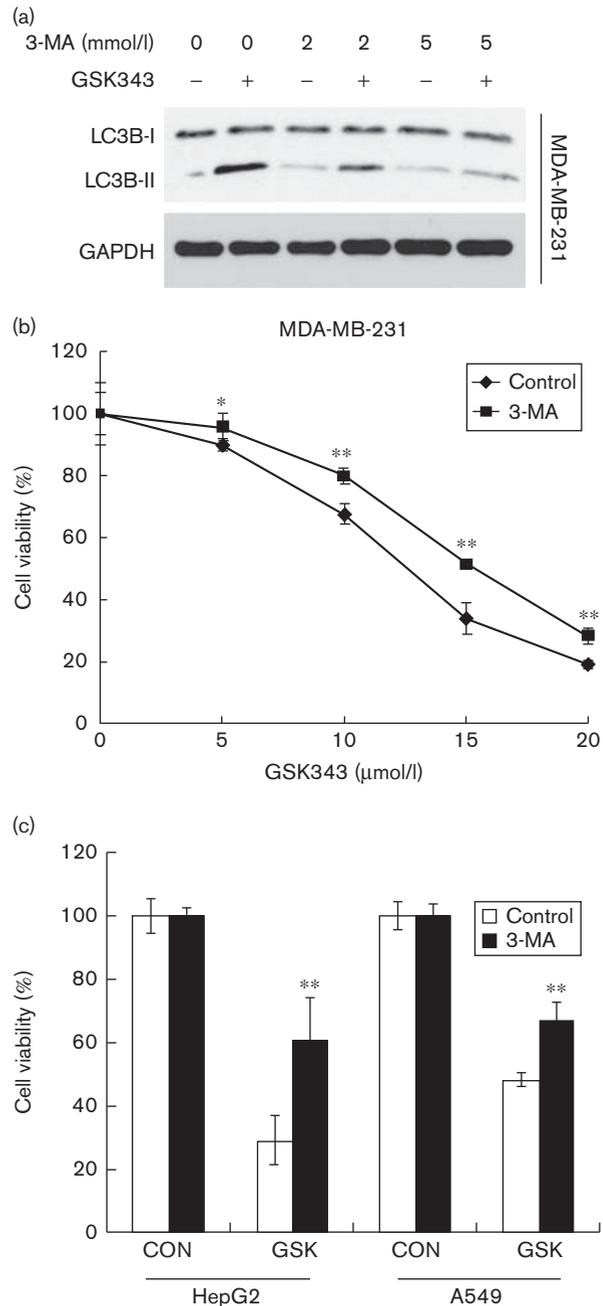
Effects of EZH2 knockdown on autophagy of cancer cells. MDA-MB-231, HepG2, and A549 cells were transiently transfected with *EZH2* siRNA for 72 h, and then whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B, p62, or GAPDH. *EZH2*, enhancer of zeste homolog 2; siRNA, small interfering RNA.

the role of autophagy in GSK343-induced cytotoxicity, MDA-MB-231 cells were pretreated with 3-MA, a class III PI3K inhibitor that blocks autophagosome formation, and were then exposed to GSK343. Western blot analysis showed that 3-MA attenuated GSK343-induced LC3-II accumulation (Fig. 5a). In addition, 3-MA also rescued GSK343-induced cytotoxicity in MDA-MB-231, HepG2, and A549 cells (Fig. 5b and c). Therefore, GSK343 induces autophagy that promotes cell death.

#### GSK343 enhances the anticancer activity of sorafenib in HepG2 cells

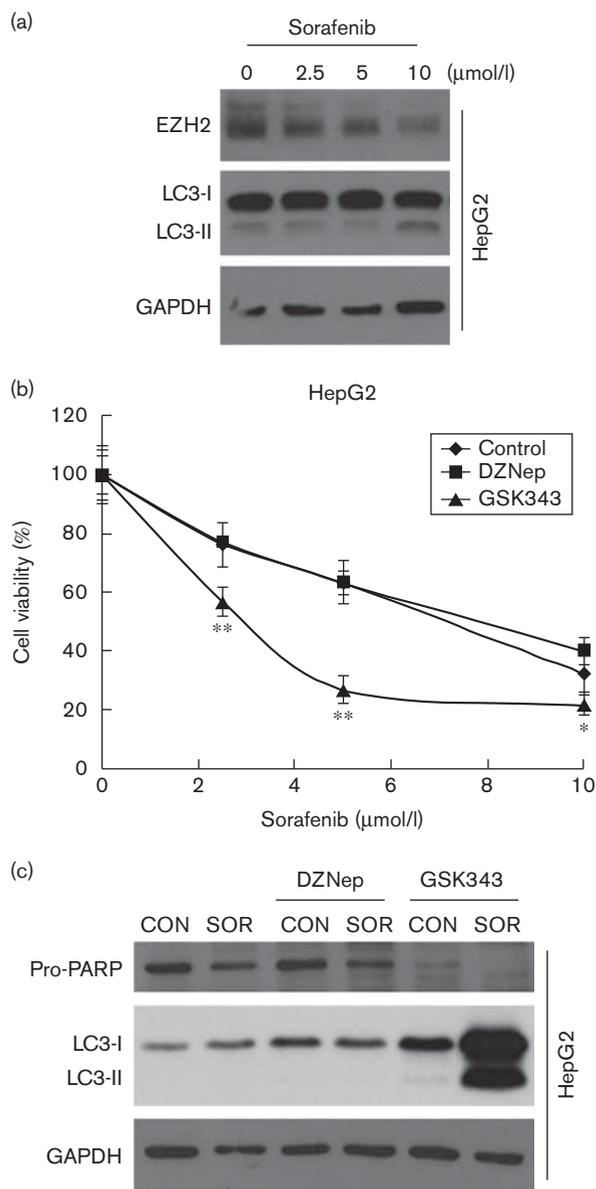
Sorafenib, a multikinase inhibitor, has been approved for the treatment of advanced hepatocellular carcinoma (HCC). Sorafenib was found to accelerate the degradation of EZH2 in HCC cells [23]. Therefore, combinational treatment with EZH2 inhibitors may enhance the anticancer activity of sorafenib in HCC cells. We also found that sorafenib dose-dependently inhibited the expression of EZH2 by HepG2 cells (Fig. 6a). However, only mild accumulation of LC3-II was induced by the treatment with 10  $\mu\text{mol/l}$  sorafenib (Fig. 6a). The MTT assay found that GSK343 but not DZNep enhanced the sensitivity of HepG2 cells to sorafenib (Fig. 6b). In addition, the cleavage of PARP was enhanced by cotreatment with GSK343 and sorafenib (Fig. 6c). In contrast, DZNep did not alter sorafenib-induced PARP cleavage (Fig. 6c). Moreover, cotreatment with GSK343 and sorafenib induced greater accumulation of LC3-II (Fig. 6c). Therefore, GSK343 but not DZNep enhanced the anticancer activity of sorafenib by promoting the autophagic cell death of HepG2 cells.

Fig. 5



Effect of 3-MA on GSK343-induced autophagy and cytotoxicity of MDA-MB-231 cells. (a) MDA-MB-231 cells were pretreated with 2 and 5 mmol/l 3-MA for 1 h and then exposed to 10  $\mu\text{mol/l}$  GSK343 for 24 h. Whole-cell lysates were subjected to a western blot analysis using antibodies against LC3B or GAPDH. (b) MDA-MB-231 cells were pretreated with 2 mmol/l 3-MA for 1 h and then exposed to various doses of GSK343 for 72 h. Cell viability was analyzed using an MTT assay. Cell viability was analyzed using an MTT assay. \* $P < 0.05$  and \*\* $P < 0.01$  indicates significant differences between 3-MA-treated and untreated cells. (c) HepG2 and A549 cells were pretreated with 2 mmol/l 3-MA for 1 h and then exposed to 10  $\mu\text{mol/l}$  GSK343 for 72 h. Cell viability was analyzed using an MTT assay. \* $P < 0.05$  and \*\* $P < 0.01$  indicates significant differences between 3-MA-treated and untreated cells. 3-MA, 3-methyladenine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

Fig. 6



Effects of DZNep and GSK343 on the anticancer activity of sorafenib in HepG2 cells. (a) HepG2 cells were treated with 2.5–10 μmol/l sorafenib for 24 h. Whole-cell lysates were subjected to a western blot analysis using antibodies against EZH2, LC3B, or GAPDH. (b) HepG2 cells were treated with various doses of sorafenib in the absence and presence of 10 μmol/l DZNep or GSK343 for 72 h. Cell viability was analyzed using an MTT assay. \* $P < 0.05$  and \*\* $P < 0.01$  indicates significant differences between drug-treated and untreated cells. (c) HepG2 cells were treated with 5 μmol/l sorafenib in the absence and presence of 10 μmol/l DZNep or GSK343 for 72 h. Whole-cell lysates were subjected to a western blot analysis using antibodies against PARP1, LC3B, or β-actin. DZNep, 3-deazaneplanocin A; EZH2, enhancer of zeste homolog 2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide.

## Discussion

Small-molecule-based inhibition of EZH2 was developed recently as an effective mechanism for therapeutic interventions against hematologic and solid tumors

[9,24]. DZNep, the first identified EZH2 inhibitor, was reported to deplete cellular levels of PRC2 components including EZH2, SUZ12, and EED, and then inhibit the trimethylation of H3K27 [6]. Recently, the discoveries of several activity-based inhibitors, such as EPZ005687, EI1, GSK126, and GSK343, represent important advances in the development of EZH2 inhibitors [8–11]. These inhibitors share a common pyridone indazole/indole scaffold and compete with the cofactor SAM to bind to EZH2. Our results show that GSK343 exerted more potent anticancer activity than DZNep, which may have been because of DZNep indirectly depleting EZH2 protein expression by inhibiting SAH [6]. In addition, knockdown of *EZH2* expression by siRNA was insufficient to induce autophagy, suggesting that inhibition of EZH2 is not required for GSK343-induced autophagy.

Our results show for the first time that SAM-competitive EZH2 inhibitors, including GSK343 and UNC1999, induce autophagy of cancer cells. SAM is generated from methionine through the catalytic activity of SAM synthetase. SAM acts as a methyl donor for numerous methyltransferase enzymes that utilize SAM as a substrate [25]. Therefore, it is possible that competition with SAM by GSK343 and UNC1999 may play a role in autophagy. A recent study showed that both methionine and SAM inhibit autophagy and promote growth in yeast [26]. Whether these phenomena also occur in mammalian cells is still unclear. Further studies should be carried out to examine whether induction of autophagy is a general effect of SAM competitors.

The basic cellular function of autophagy in eukaryotic cells is required to achieve a balance between protein synthesis and organelle biogenesis versus protein degradation and organelle turnover; thus, autophagy acts as a cytoprotective mechanism under normal circumstances. However, in several scenarios, autophagy constitutes a stress adaptation method that avoids cell death, whereas in other situations, autophagy represents an alternative pathway to cellular death [19]. In the present study, inhibition of autophagy by 3-MA protected against GSK343-induced cell death, suggesting that GSK343 induced autophagic cell death.

HCC is the fifth most common cancer and the third leading cause of cancer-related mortality worldwide [27]. Currently, surgical resection and liver transplantation are considered as the main curative treatments for HCC. However, only 15–25% of patients with HCC are eligible for curative treatments, and there is no reliably effective therapy for patients with advanced or metastatic disease. HCC is a relatively chemoresistant tumor and is highly refractory to cytotoxic chemotherapy. A number of chemotherapeutic, hormonal, and other drugs have been evaluated in clinical trials, but none of these agents or combinations has been shown to have significant efficacy [28]. Molecular-targeted agents are considered as new

treatment options. The multikinase inhibitor, sorafenib, was approved for advanced HCC in 2007 [29]. However, sorafenib monotherapy may be insufficient to achieve satisfactory results in HCC patients as it confers less than 3 months of actual gain in survival in both western and Asian populations [29,30]. Therefore, there is still an urgent need to develop effective therapeutic strategies for HCC. Our study showed that GSK343 enhanced the anticancer activity of sorafenib, which may shed light on the development of combinational therapy using sorafenib and EZH2 inhibitors.

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### Conflicts of interest

There are no conflicts of interest.

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