Enhancer of zeste homolog 2 (EZH2) is an epigenetic enzyme that mediates gene silencing through tri-methylation of histone H3 lysine 27 (H3K27-me3). Because overexpression of EZH2 in tumors is frequently found, its inhibition has been viewed as a novel anticancer strategy. Numerous small-molecule EZH2 inhibitors have been developed in recent years. S-adenosyl-L-homocysteine (SAH) hydrolase inhibitor, such as 3-Deazaneplanocin A (DZNep), and S-adenosyl-L-methionine (SAM)-competitive inhibitor, such as GSK343, represent two major types of EZH2 inhibitors. DZNep depletes EZH2 protein through the proteasome-dependent pathway. GSK343 directly inhibits the enzyme activity through competing the co-factor SAM. Our results demonstrate that GSK343, but not DZNep, induces apoptosis and autophagic cell death and enhances drug sensitivity in cancer cells. Our study shows, for the first time, that SAM-competitive EZH2 inhibitors are potent autophagy inducers, representing a novel anticancer mechanism for EZH2 inhibitors. Although autophagy is generally seen as a cytoplasmic event, recent studies reveal a transcriptional and epigenetic network that regulates autophagy. In our study, EZH2 seems not to be sufficient to initiate autophagy. However, our results provide some clues that support the promoting role of EZH2 in autophagy, which will be discussed in the highlight.

**Keywords:** autophagy; EZH2; S-adenosyl-L-methionine (SAM); cancer

SAM-competitive inhibitors, such as EPZ005687, EI1, GSK126, GSK343 and UNC1999, are developed and have been demonstrated to selectively kill lymphoma cells carrying EZH2-activating mutations [9-13].

Autophagy is a process in which cells self-digest their own organelles and macromolecules to generate energy; therefore, it functions as a temporary survival mechanism in response to starvation [14]. However, long-term nutrient deprivation eventually results in excess self-digestion and autophagic cell death [15]. Although autophagy is triggered by a specific set of cytoplasmic events, accumulating evidences have shown that nuclear events, including transcriptional and epigenetic programs, are also an essential regulator of this process [16, 17]. For the aspect of epigenetic regulation of autophagy, the histone methyltransferase G9a, that catalyzes the dimethylation of histone H3 lysine 9 (H3K9-me2), directly represses the expression of autophagy-related genes [18]. In addition, induction of autophagy is coupled to downregulation of the histone H4 lysine 16 (H4K16) acetyltransferase hMOF [19]. These studies suggest that changes in specific histone post-translational modifications initiate a regulatory feedback loop during autophagy through the transcriptional regulation of autophagy-related genes, which determines the cell fate (life or death) upon autophagy induction [19].

The above findings inspire us to investigate the role of EZH2 in autophagy. Our results have shown that the SAM-competitive EZH2 inhibitor (GSK343), but not SAH hydrolase inhibitor (DZNep), induce autophagic cell death in cancer cells [20]. The different effects of GSK343 and DZNep suggest that EZH2 inhibition is dispensable for the induction of autophagy. Indeed, siRNA-mediated knockdown of EZH2 was not sufficient to induce autophagy [20]. However, we observe that both DZNep and EZH2 siRNA can inhibit the expression of p62/SQSTM1 that is known as cargo receptors and can be degraded by autophagy [21, 22]. These results indicate that EZH2 may accelerate the autophagic flux. EZH2 could recruit class I histone deacetylase (HDAC),
including HDAC1 and HDAC2, to repress transcription cooperatively [23]. Selective inhibition of HDAC1 is known to induce autophagy [24]. In addition, our previous studies also show that HDAC inhibitors promote autophagic cell death in cancer cells, and, like DZNep, pan-HDAC inhibitors can deplete the protein expression of EZH2 [25, 26]. These findings suggest that inhibition of HDAC and EZH2 may cooperatively regulate autophagy, which awaits further investigations.

Although the rapid progress of EZH2 inhibitors in cancer therapy, their mechanisms of action to kill cancer cells are largely unclear. The apoptotic effect of DZNep in cancer cells is only partially related to its EZH2-depleting activity [7]. A SAM-competitive EZH2 inhibitor, E11, induces cell cycle arrest and apoptosis in EZH2-mutant B-cell lymphomas [11]. Our study shows that two SAM-competitive EZH2 inhibitors, GSK343 and UNC1999, exerts more potent anticancer activity than DZNep. In addition, GSK343 and UNC1999, but not DZNep, induced apoptosis and autophagic cell death in cancer cells [20]. Although apoptosis initially contributes to the response of cancer therapy, subsequent acquisition of apoptosis resistance will occur. Therefore, targeting to the alternative cell death pathways is a potential strategy to overcome drug resistance [27]. The activation of autophagy represents a novel cancer treatment target for the SAM-competitive inhibitors of EZH2.

Taken together, our study shows, for the first time, that SAM-competitive and SAH hydrolase-dependent inhibitors of EZH2 exert differential effects toward cancer cells. SAM-competitive inhibitors are potent inducer of autophagy in cancer cells. In addition, our study implies the promoting role of EZH2 in autophagy, which awaits further investigations.

**Conflicting interests**

All authors declare that there is no conflict of interest.

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**References**

17. Fullgrabe J, Klionsky DJ, Joseph B. The return of the nucleus:


