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Mini-review

Naturally occurring anti-cancer agents targeting EZH2

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Natural products are considered as promising tools for the prevention and treatment of cancer. The enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase unit of polycomb repressor complexes such as PRC2 complex that has oncogenic roles through interference with growth and metastatic potential. Several agents targeting EZH2 has been discovered but they often induce side effects in clinical trials. Recently, EZH2 has emerged as a potential target of natural products with documented anti-cancer effects and this discloses a new scenario for the development of EZH2 inhibitory strategies with agents with low cytotoxic detrimental effects. In fact, several natural products such as curcumin, triptolide, ursolic acid, sulforaphane, davidinin, tanshinodil, gambogenic acid, berberine and Alcea rosea have been shown to serve as EZH2 modulators. Mechanisms like inhibition of histone H3K4, H3K27 and H3K36 trimethylation, down-regulation of matrix metalloproteinase expression, competitive binding to the S-adenosylmethionine binding site of EZH2 and modulation of tumor-suppressive microRNAs have been demonstrated to mediate the EZH2-inhibitory activity of the mentioned natural products. This review summarizes the pathways that are regulated by various natural products resulting in the suppression of EZH2, and provides a plausible molecular mechanism for the putative anti-cancer effects of these compounds.

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Introduction

Epigenetic regulation of gene expression plays a key role in tumorigenesis [1]. Chromatin configuration at the promoter and enhancer regions can change the accessibility of transcription factors to bind to specific gene sequences to silence or enhance the expression of genes association with tumor suppression or cell cycle [2]. DNA methylation at CpG islands, and histone modification by methylation, acetylation ubiquitylation, and phosphorylation are two fundamental mechanisms of epigenetic regulation that are implicated in oncogenesis [3]. Among the histone modifications associated with cancer, methylation of histone lysine residues have gained much importance in the recent years [4]. Polycomb repressive complex 2 (PRC2), is one such methyltransferase that methylates lysine-27 of histone H3 (H3-K27). PRC2 consists of the core subunits Extraembryonic Ectoderm Development (EED), Suppressor of Zeste 12 (SUZ12), and the Enhancer of Zeste 2 (EZH2) methyltransferase [5]. Among histone methyltransferases, EZH2 has emerged as a distinct molecule whose overexpression and functional modifications have been implicated in various types of cancer [6]. It is noteworthy that EZH2 is one of the most investigated histone methyltransferase with several thousands of papers

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on cancer. The clinical role of EZH2 has been widely proven. For example, EZH2 is an independent predictor of survival in breast cancer [7]. In fact, EZH2 overexpression has been linked to poor prognosis [8] and to aggressive breast cancer [9], associated with difficult-to-treat basal or triple negative breast cancer. Moreover, it is specifically involved in the regulation of oncogenic potential having a direct role in malignant phenotype. This is an advantage if compared to other histone methyltransferases that are implicated in the regulation of multiple phenotypes. Interestingly, small molecules that effectively inhibit the enzymatic activity of EZH2 have shown preliminary evidence of clinical responses in early phase trials. Nutrients and natural compounds are known to interact with epigenetic phenomenon and modify gene expression at transcriptional level [10]. In this review, we comprehensively discuss the role of natural anticancer products in the suppression of EZH2, and provides a plausible molecular mechanism for the putative anti-cancer effects of these compounds.

**EZH2: biological role and relevance to cancer**

EZH2, a polycomb group protein homolog of Drosophila enhancer of zeste, is a histone methyltransferase unit of PRC2 complex. The latter complex is composed of H3K27 methyltransferase (KMT), a suppressor of zeste 12 (SUZ12), embryonic ectoderm development (EED), and retinoblastoma (Rb)-associated protein 46/48 (RbAp46/48) and regulates gene silencing through methylation of histone 3 at lysine 27 (H3K27) residue [11]. Accordingly, EZH2-dependent methylation (H3K27) changes chromatin configuration and leads to chromatin condensation and consequent gene silencing [12,13].

Deregulation of this histone modification can lead to epigenetic silencing of tumor suppressor genes thus leading to tumorigenesis [14]. Apart from H3K27, EED isoform 2 (Eed2) and NAD-dependent histone deacetylase Sirt1 specifically associated within the PRC4 which is needed for methylating linker histone H1 (H1K26) [15]. This modification is specific for cancer and undifferentiated embryonic stem (ES) cells. Moreover, Jarid2, belonging to Jumonji family of histone demethylases without enzymatic activity, binds DNA with a relatively high affinity for GC rich sequences recruiting PcG proteins to target genes [16]. Components of PRC2 are required for embryonic development and loss of EZH2 gene is associated with a block in B- and T-cell differentiation. Moreover, EZH2 acts as an oncogene, as it is overexpressed in many solid cancers in both advanced and metastatic diseases [17]. It was demonstrated that an activating mutation in the SET domain of EZH2 occurs in 7% of large follicular lymphomas, 22% of diffuse B cell lymphomas and 3% of melanomas [18]. These mutations confer a “gain of (methyltransferase) function” to EZH2 determining its oncogenic activity. Studies in immortalized HTLV-1 infected T cells and from cells from adult T cell leukemia (ATL) showed that EZH2 is involved in miR-31 downregulation in an epigenetic fashion, which in turn activates NF-κB and resistance to apoptosis. Similarly, in HTLV1-infected cells Tax protein binds EZH2 increasing its activity [19]. In hepatoblastoma virus-dependent hepatocellular carcinoma, the viral protein YY1 can recruit EZH2 to DNA inducing the silencing of 5 miRNAs with NFκB suppressive function [20]. EZH2 over expression and silencing of the tumor suppressor miR-31 was also found in prostate cancer and multiple myeloma patients [11,21]. In this light, different roles have been suggested for polycomb proteins such as stem cell maintenance, imprinting, X-inactivation, differentiation, and proliferation [22,23]. Beyond the interaction of EZH2 with NFκB pathway, EZH2 oncogenic activity can also be mediated through the activation of Akt and the consequent increase of STAT3 activity [24]. In addition to “gain of function” mutations, “loss of function” mutations of EZH2 were also found in a model of myeloid malignancy. It was, in fact, demonstrated that these mutations induces the activation of HOXA9 genes mediating the self-renewal of progenitor cells predisposing animal to different hematological neoplasms [25]. Regulation of cell cycle is another mechanism of oncogenic regulation induced by EZH2. EZH2 was shown to be downstream of the retinoblastoma-E2F pathway and activate proliferative genes and E2F-regulated proliferation [26]. InK4a/Arf and E-cadherin, FOXC1 and DNA repair pathways are other check points regulated by EZH2 [26,27].

Some oncogenic functions of PRC2 and EZH2 are not associated to the methylating functions of the latter. In fact, in castration-resistant prostate cancer, EZH2 acts as a coactivator for critical transcription factors including the androgen receptor [28]. Another non-conventional function of EZH2 is the methylation of non-histone proteins. In fact, the methylation of these proteins can facilitate the recognition by the ubiquitination machinery driving their degradation [29,30].

Additional findings have demonstrated that high EZH2 expression was associated to aggressiveness and poor clinical outcome of different human cancers, including breast, gastric, bladder, endometrial, hematopoietic, ovarian cancers and melanoma [31]. Moreover, it was reported that increased EZH2 expression in pediatric neoplasms such as soft tissue sarcomas and T cell lymphomas [32]. Estrogenic leukemia was correlated with a poor clinical outcome [32,33].

On the basis of these findings, different pharmacological approaches have been designed to target EZH2 and to inhibit tumor cell proliferation.

**EZH2 as a therapeutic target**

The first EZH2 inhibitor widely explored for experimental studies is 3-deazaneplanocinA (DZNep), a cyclopentanyl analog of 3-deazadenosine that potently interferes with S-adenosyl-l-homocysteine hydrolase (SAH) [34]. In this light, the effects of DZNep on inhibition of histone methylation are not specifically restricted to EZH2. However, treatment with DZNep induces significant antitumor activity in various cancer types occurring together with inhibition of PRC2, and removal of H3K27me3 [35]. Important limitations of DZNep are its very short plasma half-life and its toxic effects in preclinical models [36].

After high-through-put screening of different inhibitors based upon SET structure of EZH2, several more specific and potent inhibitors have been recognized. EPZ005687 binds to wild-type and Y641-mutant EZH2 with greater than 500-fold selectivity for EZH2 compared to 15other human protein methyl transferases. EPZ005687 shows dose-dependent inhibition of H3K27me3 in EZH2–wild-type and, Y641- and A677-mutant lymphoma cells as well as in cell lines of other cancer types, including breast and prostate cancer [37]. Another small-molecule EZH2 inhibitor, GSK126, inhibits both wild-type and mutant EZH2 and has greater than 1000-fold selectivity for EZH2 compared to other methyltransferases. GSK126 markedly inhibits the growth of lymphomas carrying activating EZH2 mutations in vivo [38]. A third independent SAM-competitive inhibitor, E11, inhibits both wild-type and mutant EZH2 inhibiting H3K27me2/3 levels without affecting EZH2 protein levels. These effects were paralleled by cell growth inhibition, cell cycle arrest and apoptosis in cells carrying EZH2 mutations [39]. Finally, UNC1999 is the first orally bioavailable inhibitor highly selective for wild-type EZH2 and the EZH2 Y641 mutant [40]. Similarly, EPZ-6438 was developed from EPZ005687 with improved pharmacokinetic properties including good oral bioavailability [41]. EPZ005687showes dose-dependent inhibition of H3K27me3 in wild-type and, Y641- and A677-mutant lymphoma cells as well as in cell lines of other cancer types, including breast and prostate cancer [37]. Another small-molecule EZH2 inhibitor, GSK126, inhibits both wild-type and mutant EZH2 and has greater than 1000-fold selectivity for EZH2 compared to other methyltransferases. GSK126 markedly inhibits the growth of lymphomas carrying activating EZH2 mutations in vivo [38]. A third independent SAM-competitive inhibitor, E11, inhibits both wild-type and mutant EZH2 inhibiting H3K27me2/3 levels without affecting EZH2 protein levels. These effects were paralleled by cell growth inhibition, cell cycle arrest and apoptosis in cells carrying EZH2 mutations [39]. Finally, UNC1999 is the first orally bioavailable inhibitor highly selective for wild-type EZH2 and the EZH2 Y641 mutant [40]. Similarl, EPZ-6438 was developed from EPZ005687 with improved pharmacokinetic properties including good oral bioavailability [41]. EPZ005687 showes dose-dependent inhibition of H3K27me3 in wild-type and, Y641- and A677-mutant lymphoma cells as well as in cell lines of other cancer types, including breast and prostate cancer [37]. Another small-molecule EZH2 inhibitor, GSK126, inhibits both wild-type and mutant EZH2 and has greater than 1000-fold selectivity for EZH2 compared to other methyltransferases. GSK126 markedly inhibits the growth of lymphomas carrying activating EZH2 mutations in vivo [38]. A third independent SAM-competitive inhibitor, E11, inhibits both wild-type and mutant EZH2 inhibiting H3K27me2/3 levels without affecting EZH2 protein levels. These effects were paralleled by cell growth inhibition, cell cycle arrest and apoptosis in cells carrying EZH2 mutations [39]. Finally, UNC1999 is the first orally bioavailable inhibitor highly selective for wild-type EZH2 and the EZH2 Y641 mutant [40]. Similarly, EPZ-6438 was developed from EPZ005687 with improved pharmacokinetic properties including good oral bioavailability [41].
PHYTOPHARMACEUTICALS WITH INHIBITORY EFFECTS ON EZH2

Despite the great efforts of pharmacological research to find specific inhibitors of EZH2, consolidated strategies to antagonize the activity of this protein have not yet been found. On the other hand, several natural compounds, often derived by traditional Eastern Medicine, show an inhibitory activity against EZH2. The advantages of these substances are their relatively high oral bioavailability and the poor side effects. In this view, we will describe the most important ones and their anti-cancer activity based upon EZH2 inhibition.

Curcumin

Curcumin, a polyphenolic natural component, is expressed in different kinds of herbs, mainly in the rhizomes of the East Indian plant Curcuma longa Linn (turmeric). Rhizomes of C. longa usually contain a mixture of three major curcuminoids including (in order of their abundance) curcumin, demethoxycurcumin and bisdemethoxycurcumin, which collectively contribute to the medicinal properties and also to the yellow color of turmeric [43,44]. Turmeric or turmeric extracts have been widely used not only as flavoring agents but also as well-established medicinal agents in Eastern medicine including Ayurvedic, Chinese and Iranian medicine [45]. Moreover, numerous pre-clinical and clinical studies have unveiled a multitude of pharmacological and biological effects of curcumin including anti-tumor [46–48], anti-dyslipidemic [49–52], anti-inflammatory [53–55], immunomodulatory [56–60], analgesic [61,62], anti-depressant [63,64], anti-oxidant [65–67], anti-pruritic [67], hypouricemic [68] and hepatoprotective [69,70] activities. In spite of its superb pharmacological effects, clinical application of curcumin has been argued due to its relatively low systemic bioavailability [71]. However, a new analog of curcumin, difluorinated-curcumin (CDF), has been recently developed showing to possess potent anti-cancer effects and higher metabolic stability compared with curcumin, thereby leading to higher systemic bioavailability and extended circulating half-life [72].

Over the years, several preclinical studies have proposed curcumin as a natural product for both cancer prevention and treatment [73]. Moreover, it can inhibit cell proliferation in different human cell lines in vitro and prevent and treat malignant diseases in vivo [74]. In spite of the well-established anti-tumor effects of curcumin, the exact molecular mechanisms through which curcumin prevents cell proliferation and induces chemo-sensitizing effects in different tumor cells is not fully understood [75,76].

Curcumin suppresses EZH2 expression through different mechanisms in a variety of cancers [74,77]. It reduces the expression of EZH2 and suppresses EZH2-mediated H3K27 methylation in human breast cancer cells. Moreover, curcumin affects the accumulation of cells in the G1 phase and reduces the cell number in S phase [74]. According to another study, curcumin inhibits both lung cancer cell growth (arresting the cells in G2/M phase) and metastatization through down-regulation of EZH2 expression mediated by miR-101 and miR-let 7c. This decrease of EZH2 expression was paralleled by down-regulation of NOTCH1 suggesting that anti-tumor effect of curcumin was due, at least in part, to the inhibition of EZH2/NOTCH1 pathway, considered as an important target in lung cancer [77]. In fact, NOTCH1 has been associated to the development of several tumors and is considered a marker of stemness [78]. Several reports have shown the contribution of curcumin in the sensitization of cancer cells to chemotherapy drugs [76,79]. In colorectal cancer (CRC), resistance to chemotherapy has been considered as a major hurdle to treat malignant patients [90]. 5-fluorouracil (5FU) is one of the mainstay in the treatment of CRC [80] and both epithelial–mesenchymal transition (EMT) with loss of E-cadherin expression and stemness are involved in the resistance to 5FU [91,92]. Studies using in vitro generated chemoresistant CRC cell lines indicated the involvement of curcumin in inducing epithelial differentiation and increasing EMT-suppressive miRNAs expression. In this report, curcumin was able to inhibit both EMT and PRC at the same time and this effect was paralleled by a significant increase of miR-200c. The anti-cancer potential of curcumin in vivo is also supported by the fact that curcumin, CDF and EMT thus suggesting that the inhibitory effects of curcumin on EZH2 and EMT were likely due to the regulation of mir-200c expression. Moreover, in these experimental conditions, curcumin enhanced the anti-proliferative effects of 5FU in chemoresistant cell lines both in vitro and in vivo [86]. Another compelling study indicates that curcumin has potent anti-proliferative effects on both the established tamoxifen-resistant MCF-7 cells and their wild type counterparts. In these experimental conditions, curcumin was able to antagonize the hormone-resistant phenotype in endocrine-resistant cells and potentiate the anti-proliferative effects of tamoxifen in parental cells. The molecular bases of these effects were suggested to be the arrest of cells in G2/M phase and the decreased expression of molecules involved in cell cycle progression. Moreover, the authors provided evidence on the concomitant decrease of EZH2 expression induced by curcumin [93,94].

Likewise curcumin, CDF, a natural anti-tumor agent derived from curcumin, is able to regulate cell migration, survival and invasion, cancer stem cell self-renewal, and EZH2 expression. The data showed that treatment of pancreatic cancer cells with CDF significantly suppresses the expression of cancer stem cells markers, EZH2 as well as markers for drug resistance and tumor metastasis. Emerging evidence has demonstrated that miRNAs play a critical role in the regulation of cancer stem cell markers, including pancreatic cancer[ref]. The let-7 family of miRNAs are strong regulators of cancer stem cell genes and are down regulated in pancreatic tumors [95]. CDF treatment was found to down-regulate EZH2 and induce the expression of tumor-suppressive miRNAs including let-7 family, miR-200, miR-101 and miR-26a. A negative mutual feedback loop between expression of miRNA-101 and EZH2 was observed wherein the inactivation of EZH2 caused the upregulation of miRNAs-101. Similarly, the results of in vivo treatment of CDF in a xenograft model of human pancreatic cancer was consistent with in vitro findings, displaying an increased expression of tumor-suppressive miRNAs and decreased levels of cancer stem cell genes. The authors concluded that CDF inhibited tumor metastasis and invasion by mediating an EZH2-miRNAs regulatory system. CDF also attenuated cancer stem cell expression thus overcoming an important mechanism of therapeutic resistance [20]. It was also found that under hypoxic conditions, the anti-tumor activity of CDF was mediated by targeting the hypoxia-mediated miRNAs, with concomitant down regulation of EZH2...
Co-administration of DZNep and EGCG inhibited S-adenosylhomocysteine hydrolase and S-adenosyl-L-methionine-dependent methyltransferase activity [98]. Consequently, EZH2 methyltransferase activity is also reduced as a result of limited availability of methyl groups [35,107,109].

**Triptolide**

Triptolide, a diterpenoid triepoxide, is a principal bioactive ingredient of extracts from Chinese Herb *Tripterix hypophoroides Hook F* (TWHF) that has been used in traditional Chinese medicine for hundreds of years [110]. Recently, it was demonstrated that triptolide has both *in vitro* and *in vivo* anti-cancer activity on a series of human cancers of different histologies and has focussed the attention on triptolide in cancer research [111]. In multiple myeloma cells, anti-carcinogenic properties of triptolide have been shown to be mediated by the regulation of histone methylation. Moreover, triptolide treatment decreased the level of trimethylated H3K27 and this effect was paralleled by the reduced mRNA and protein expression of EZH2. Overall, these effects led to cell cycle arrest in G2/M phase, induction of caspase-dependent apoptosis and leading to apoptosis and growth suppression in multiple myeloma cells [112]. There is also evidence showing the anti-myeloma activity of triptolide through downregulation of both histone H3K9 and H3K27 methylation. H3K9 methylation is a hallmark of histone methyltransferase SUV39H1 function, causing formation of a binding site for heterochromatin protein 1 (HP1), the latter involved in chromatin packaging, heterochromatin formation and gene silencing [113]. In this latter study, triptolide was shown to reduce SUV39H1 and EZH2 at mRNA and protein levels in a dose-dependent manner, and to induce G0/G1 cell cycle arrest and mitochondria-dependent apoptosis [110]. EZH2 can trigger prostate tumorigenesis and metastasis [114]. In prostate cancer (PCa) cells, EZH2 treatment displayed growth inhibition paralleled by EZH2 inhibition (at both mRNA and protein level) that was dose- and time-dependent [115].

**Ursolic acid**

Ursolic acid (UA), a pentacyclic triterpene isolated in abundance from the peels of *Malus pumila Mill* has been reported to possess a wide range of pharmacological properties, including anti-cancer activity via a variety of biological functions involving apoptosis, chemos- and radiotherapy sensitization, and mitigation of tumor invasion and metastasis [106–108]. It was recently reported that UA inhibits the growth of non-small cell lung cancer (NSCLC) cells through phosphorylation and induction of SAPK/JNK, a member of the MAPK family. This effect was accompanied by suppression of SP1 (a ubiquitous transcriptional factor) which, in turn, led to the inhibition of DNM1 and EZH2 expression. Thus, the SAPK/JNK/EZH2/DNM1 signaling cascades contributes to the inhibitory effects of UA on both EZH2 and DNM1 [120]. Findings from a recent study suggest that UA exerts its anti-proliferative effects through induction of caspase-dependent apoptosis and cell cycle arrest in G2/M phase in osteosarcoma cells. Repression of mRNA and protein expression of EZH2 was shown as the main mechanism for the above-mentioned inhibitory effects of UA on osteosarcoma cells [121].

**Sulforaphane**

Epidemiological studies indicate that consumption of cruciferous vegetables can reduce epithelial cancer. Sulforaphane [SFN; 1-isothiocyanato-4-(methylsulfinyl) butane] is an abundant bioactive isothiocyanate found in broccoli and broccoli sprouts and is an associated with elevated levels of Bmi-1, EZH2 and SUZ12 [106,107].
important chemopreventive agent [123]. In melanoma cells, SFN treatment reduced EZH2 and H3K27me3 levels. Furthermore, SFN treatment suppressed the levels of two other demethylases (JMJD3 and UTX) to reduce H3K27me3 [122]. The above-mentioned effects of SFN were paralleled by the following biological effects: i) G2/M phase accumulation and cyclin B1, cyclin A, cyclin dependent kinases 1 and 2 reduction and p21Cip1 expression increase: ii) induction of apoptosis paralleled by increased cleavage of procaspase 3, 8, and 9 and enhanced PARP cleavage. Interestingly, forced expression of the Bmi-1 polycomb protein in SCC-13 cells reversed these effects [124]. EZH2 is overexpressed in melanoma stem cells (MSC) and contributes to MSC sphere formation and to the invasive and metastatic potential of melanoma cells. SFN has been shown to suppress MCS cell survival and reduce EZH2 expression. On the other hand, induced expression of EZH2 partially reversed SFN suppression of MCS both in vitro and in vivo. SFN treatment reduced H3K27me3 formation and matrix MMP expression and increased TIMP3 expression and apoptosis [123].

Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene) is a natural polyphenolic flavonoid present in red grape, red wine, and other plant species with anti-oxidant, anti-inflammatory, and anti-tumor activities [126]. It was found that the miR-137 upregulation reduces EZH2 levels in neuroblastoma cells treated with resveratrol and this effect mediates the anti-proliferative activity of the latter. Inhibition of miR-137 expression diminishes the EZH2 reduction in neuroblastoma cells triggered by resveratrol [127]. It was also reported that resveratrol has anti-myogenic activity in oral fibroblasts which was mediated by the induction of either EZH2 or miR-200c, leading to the trimethylation of H3K27 and a consequent transcriptional suppression of the zinc finger E-box binding homeobox 1 (ZEB1) promoter [128].

Davidiin

Polygonum capitatum is an Asian medicinal plant with antibacterial, anti-tumor and anti-inflammatory activities [129]. Davidiin, a phytochemical extracted from P. capitatum, has been shown to inhibit hepatocellular carcinoma (HCC) growth and possess anti-cancer properties. Davidiin induces apoptosis through increased cleavages of both caspase-3 and poly ADP ribose polymerase (PARP). In addition, davidiin directly downregulates EZH2 protein levels and enhances proteasome-dependent degradation of EZH2 in HCC. Decreased levels of EZH2 protein were associated with reduced H3K27 trimethylation in HCC cells. Surprisingly, other PCg core proteins were unaffected by davidiin treatment, suggesting EZH2 as the major therapeutic target of davidiin in HCC [130].

Tanshindiolis

The root of Salvia miltiorrhiza (Danshen) has a long history of use in the Traditional Chinese Medicine [131]. Tanshminones are the major class of active components present in S. miltiorrhiza roots and have an abietane-type diterpene structure. Nevertheless, minor components such as tanshindiolis have also been found to possess biological activities. Tanshindiolis B and C were reported to inhibit EZH2 methyltransferase activity with IC_{50} values of 0.52 μM and 0.55 μM, respectively. Among the various cell lines treated with tanshindiolis C, Pfeiffer cells harboring EZH2 A677G activating mutation displayed the highest sensitivity with an IC_{50} value of 1.5 μM, while U-2932 and Daudi lymphoma cell lines were found to be less sensitive (IC_{50} values of 9.5 μM and 10.6 μM, respectively). It was indicated that tanshindiol C penetrates to Pfeiffer cell cells and suppresses H3K27 trimethylation. Tanshindiol C can bind to the S-adenosylmethionine (SAM) binding site of EZH2 and act as a competitive inhibitor of SAM, thereby suppressing EZH2 activity [132].

Gambogic acid and methyl jasmonate

Gambogic acid (GA) is a natural product with known anti-cancer properties and is extracted from the resin secreted from the Garcinia hanburyi tree in Southeast Asia [133]. GA has been indicated to inhibit various types of cancer such as prostate cancer, gastric carcinoma and lung carcinoma both in vitro and in vivo [134,135]. GA exerts its anti-cancer effects through different mechanisms including induction of apoptosis and down-regulation of oncogenes [136]. Further to GA, jasmonates – which are plant stress hormones — and their synthetic derivatives exhibit anti-cancer activities both in vitro and in vivo, and can enhance the effects of anti-cancer agents [137]. Particularly, among the naturally occurring jasmonates, methyl jasmonate (MJ) has been considered as the most active in terms of apoptosis-inducing effects [138]. In bladder cancer, high expression of EZH2 has been shown to be associated with the histological grade and invasiveness of the disease [139]. In an experiment to explore the synergistic effects of GA and MJ in bladder cancer cell lines, MJ enhanced GA-induced apoptosis by enhancing the activities of caspase-3 and caspase-9, and inhibiting the expression of X-linked inhibitor of apoptosis protein (XIAP). The underlying mechanism for this effect was related to the down-regulation of EZH2 expression by the tumor-suppressor miR-101. It was demonstrated that miR-101 can directly trigger EZH2 in bladder cancer, and co-administration of GA and MJ up-regulates this miRNA in bladder cancer cells [140].

Alcea rosea

Alcea rosea (AR) is an ornamental plant belonging to Malvaceae. It is popularly known as Holyhock and is widely grown in gardens and parks in the Southern Europe and Asia. Several pharmacological studies have reported that this plant possesses anti-inflammatory, anti-bacterial and analgesic effects [141]. In Iranian traditional medicine, the roots of AR are used as medicine for a wide range of ailments, including bronchitis, diarrhea, constipation, inflammation, severe coughs and angina [141]. The AR seed extract (AR extract) inhibited proliferation and colony formation via triggering an apoptotic effect in colon cancer cells. In addition, AR extract induced cell cycle arrest in the G0/G1 phase and inhibited the growth of the cancer stem cell component in the colonospheres of colon cancer cells in vitro. These effects were paralleled by in vivo inhibition of the growth of tumors derived from colon cancer cells that occurred through reduced levels of EZH2, β-catenin, CyclinD1 and Ki-67 along with reduced levels of CSC markers [142].

Berberine

Berberine, an isoquinoline alkaloid, inhibits cell growth in several types of human cancers. It has been indicated that berberine induces inhibition of EZH2 expression paralleled by inhibition of cell proliferation, G1 phase cell cycle arrest and reduction of invasive properties of esophageal cancer cells [143].

Conclusions

EZH2, an enzymatic subunit of PRC2, has been demonstrated to have a pivotal role in the development and metastasization of a great number of tumors. This has also been demonstrated by the existence of activating mutations in several cancers and by the restoration of the normal phenotype after their abrogation through
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<td>[78]</td>
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<tr>
<td>Curcumin</td>
<td>MiaPaCa-2 cells Xenograft mouse model implanted with MiaPaCa-2 cells</td>
<td>Pancreatic cancer</td>
<td>0.5 µM (in vitro) 2.5 mg/mouse/day (in vivo)</td>
<td>Reduction of cell survival, clonogenicity, pro-apoptosis and chemosensitizing effects to tamoxifen</td>
<td>Down-regulation</td>
<td>MiR-let-7a,b,c,d (+)</td>
<td>NOTCH1 (−)</td>
<td>[23]</td>
</tr>
<tr>
<td>Tanshindiol C</td>
<td>Pfeiffer, U-2932, Daudi, PC3, T98G, U87MG, and A549</td>
<td>Breast cancer, glioma, and lung cancer</td>
<td>IC50 values: Pfeiffer (1.5) U-2932 (9.5) Daudi (10.6) PC3 (4.9) T98G (6.0) U87MG (5.7) AS49 (4.2)</td>
<td>Induction of apoptosis</td>
<td>Down-regulation</td>
<td>NA</td>
<td>NA</td>
<td>[120]</td>
</tr>
<tr>
<td>Davidin</td>
<td>Hep3B, HepG2 PLC/PRF/5, and Bel7404</td>
<td>Hepatocellular carcinoma</td>
<td>40 µM</td>
<td>Induction of apoptosis, induced proteasome activation</td>
<td>Down-regulation</td>
<td>NA</td>
<td>NA</td>
<td>[129]</td>
</tr>
<tr>
<td>ECGG</td>
<td>HCT116, SW480 cells</td>
<td>Colorectal cancer cells</td>
<td>50 µM</td>
<td>Induction of apoptosis, chemosensitization to 5-FU and cell cycle arrest</td>
<td>Down-regulation</td>
<td>miR-34a (+) miR-200c (+) miR-145 (+)</td>
<td>Notch1 (−) cMyc (−) Bmi1 (−) SUZ12 (−)</td>
<td>[99]</td>
</tr>
<tr>
<td>ECGG</td>
<td>HaCaT, A431, SCC-13, MCF-7, MDA-MB-231 cells</td>
<td>Skin cancer cells</td>
<td>60 µM</td>
<td>Anti-proliferative effect, Induction of apoptosis</td>
<td>Down-regulation</td>
<td>NA</td>
<td>Bmi1 (−) SUZ12 (−) NR</td>
<td>[107]</td>
</tr>
<tr>
<td>ECGG and green tea polyphenol</td>
<td>MCC-7, MDA-MB 231 cells</td>
<td>Breast Cancer Cells</td>
<td>10 µg/mL GTP and 20 µm ECGG</td>
<td>Induction of TIMP-3, Reduction of class I HDAC protein levels (HDAC-8), increase in H3K9/18 acetylation, inhibition of MMP-2 and MMP-9 activities</td>
<td>Down-regulation</td>
<td>NA</td>
<td>Bmi1 (−) SUZ12 (−) NR</td>
<td>[102]</td>
</tr>
<tr>
<td>Epigallocatechin-3-gallate and DZNep</td>
<td>SCC-13, and A431 cells</td>
<td>Skin cancer cells</td>
<td>100 µM ECGG and 15 µM DZNep</td>
<td>Anti-proliferative effect, Induction of apoptosis and proteasome</td>
<td>Down-regulation</td>
<td>NA</td>
<td>Bmi1 (−) SUZ12 (−) NR</td>
<td>[142]</td>
</tr>
<tr>
<td>Berberine</td>
<td>KYSE450 cell</td>
<td>Esophageal cancer cells</td>
<td>—</td>
<td>Anti-proliferative effect</td>
<td>Down-regulation</td>
<td>NA</td>
<td>Receptor tyrosine kinase AXL (+) EED (+) SUZ12 (−) RING1B (−) BMI-1 (−) CIU (−) NGFR (+)</td>
<td>[142]</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>Neuro-2a (N-2a), SH-SYSY cells</td>
<td>Neuroblastoma cells</td>
<td>80 µM (Anti-proliferative effect) 20 µM (decreased EZH2)</td>
<td>—</td>
<td>Down-regulation</td>
<td>miR-137 (+)</td>
<td>NA</td>
<td>[115]</td>
</tr>
<tr>
<td>Compound</td>
<td>Cell Line Description</td>
<td>Cancer Type</td>
<td>IC50 Values (μM)</td>
<td>Effect(s)</td>
<td>miR (†) Regulation</td>
<td>Gene(s) (-)</td>
<td></td>
<td></td>
</tr>
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<td>--------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>BMF cell lines (BMF1, BMF2, and BMF3)</td>
<td>Oral submucous fibrosis (OSF)</td>
<td></td>
<td>Anti-myofibroblast activity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triptolide</td>
<td>RPM8226 cells</td>
<td>Multiple myeloma (MM)</td>
<td>40, 80, 160 mmol/L</td>
<td>Anti-proliferative effect, Induction of apoptosis</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triptolide</td>
<td>U266 cells</td>
<td>Multiple myeloma (MM)</td>
<td>40 nmol/L</td>
<td>Anti-proliferative effect, Induction of apoptosis</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triptolide</td>
<td>LNCaP and PC-3 cells</td>
<td>Prostate cancer (PCA)</td>
<td>0.1 μM</td>
<td>Anti-proliferative effect</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcea rosea</td>
<td>HCT 116 and SW480</td>
<td>Colorectal cancer</td>
<td></td>
<td>Anti-proliferative effect, Induction of apoptosis</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combination of Gambogic acid and methyljasmonate</td>
<td>T24, BIU-87 and FB cells</td>
<td>Bladder cancer cells</td>
<td></td>
<td>Anti-proliferative effect, Induction of apoptosis</td>
<td>Down-regulation</td>
<td>miR-101(†)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ursolic acid</td>
<td>U2OS and Saos-2 cells</td>
<td>Osteosarcoma cells</td>
<td>25 μM</td>
<td>Anti-proliferative effect, Induction of apoptosis</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulforaphane</td>
<td>SCC-13, A431, and HaCaT</td>
<td>Skin cancer cells</td>
<td>20 μM</td>
<td>Anti-proliferative effect, Induction of apoptosis, Proteasome induction</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulforaphane</td>
<td>A375, and WM793 cells</td>
<td>Melanoma cancer stem cells</td>
<td>20 μM</td>
<td>Reduction of cancer stem cells survival, Suppression of cell invasion and cell migration</td>
<td>Down-regulation</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Study was conducted with difluorinated curcumin (CDF); **Not reported. miR: microRNA; EMT: epithelial–mesenchymal transition; JNK: c-Jun NH2-terminal kinase; ERK: extracellular signal-regulated kinase; Epigallocatechin-3-gallate (EGCG); Jumonji domain protein 3 (JMJD3); Tetrameric peptide repeat, X protein (UTX); Matrix metalloproteinase (MMP); metalloproteinase inhibitor; Nuclear receptor-binding SET domain-containing protein (NSD1); MYND domain-containing protein 3 (SMYD3); β-2 adrenergic receptor (ADRB2); cyclin-dependent kinase inhibitor 2A (CDKN2A); aldehyde dehydrogenase 1 (ALDH1A1); double cortin-like kinase-1 (Dclk1); Zinc finger E-box binding homebox 1 (ZEB1).
anti-sense strategies. On these bases, several pharmaceutical compounds have been designed and developed and some of these have given some promising results in preliminary clinical trials. However, the traditional Eastern Medicine offers several natural compounds with anti-cancer properties at least in part mediated by EZH2-suppressing activity (Table 1). They have the advantages of safety and multi-target action and thus could be used as nutraceuticals in combination with other anti-cancer agents not having overlapping toxicity. At this regard, it has been recently demonstrated that the inhibition of EZH2 strongly synergizes with histone deacetylase (HDAC) inhibitor vorinostat in inducing growth inhibition of NSCL cells independently from epidermal growth factor receptor (EGFR) status (144). The co-treatment resulted in an accumulation of p27Kip1, decrease in cyclin A, and increased apoptotic fraction in an additive/synergistic manner. The results of this study suggest the combined use of natural compounds inhibiting EZH2 with HDAC inhibitors as the inhibition of both has a potentiating effect on tumor cell growth inhibition. The effects of some natural compounds as direct inhibitors of HDAC additionally support this conclusion. An example is the inhibition of HDAC induced by curcumin in a budding yeast that is sensitized to DNA damage (145). Moreover, SFN is an HDAC inhibitor and exerts its anti-proliferative activity in multiple cell models including keratinocytes and pancreas cancer cells (146). Based upon these considerations, the most promising agents to be investigated in clinical trials are those that have already demonstrated a synergism with conventional cytotoxic agents and with which they do not have overlapping side effects. In this regard, resveratrol, curcumin and SFN are the most promising and the most advanced also in clinical trials in the treatment of human cancers. The role of EZH2 is explored for this agent as a pivotal target responsible for their anti-cancer effects and based upon this surprising evidence the combination with EZH2 inhibitors is strongly warranted. The future directions on research with natural compounds as EZH2 inhibitors are based upon the finding of the optimal pharmaceutical interaction with other EZH2 inhibitors of synthetic origin or other methyltransferases or HDAC inhibitors. Another important area of research is the design and fabrication of delivery systems based upon nanocarriers for either the systemic delivery or the increase of their oral bioavailability in order to improve their pharmacokinetic and tumor tissue distribution properties. Therefore, a new scenario is being developed based upon the use of traditional drugs as target-directed agents or as co-adjutants in strategies aiming at EZH2 inhibition. This could be a future road to follow in order to improve anti-cancer therapy and reduce side effects. Fig. 1.

Conflict of interests

Muhammed Majeed is the CEO of Sabinsa Corporation and Sami Labs Ltd.

Uncited references

[81]; [82]; [83]; [84]; [85]; [87]; [88]; [89]; [117]; [118]; [119]; [144]; [145]; [146].

Acknowledgments

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References


A. Sahebkar, A. Mohammadi, A. Atabati, S. Rahiman, S. Tavallaie, J.A. Colacino, S.P. McDermott, M.A. Sartor, M.S. Wicha, L.S. Rozek, Tran-...


