Cellular & Molecular Biology
Cell. Mol. Biol. 2015; 61 (6): 69-84
Published online October 30, 2015 (http://www.cellmolbiol.com)
Received on October 2, 2015, Accepted on October 8, 2015.
doi : 10.14715/cmb/2015.61.6.10

Targeting post-translational modifications of histones for cancer therapy

Y-C. Hsu[1,*], Y-H. Hsieh[2,3,*], C-C. Liao[4], L-W. Chong[5], C-Y. Lee[6], Y-L. Yu[7,8✉], and R-H. Chou[7,8✉]

[1] Institute of Biomedical Sciences, Mackay Medical College, New Taipei City, Taiwan
[2] Department of Biochemistry, School of Medicine, Chung Shan Medical University, Taichung, Taiwan
[3] Clinical Laboratory, Chung Shan Medical University Hospital, Taichung, Taiwan
[4] Proteomics Research Center, National Yang-Ming University, Taipei, Taiwan
[5] Division of Hepatology and Gastroenterology, Department of Internal Medicine, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan
[6] School of Chinese Medicine for Post-Baccalaureate, I-Shou University, Kaohsiung, Taiwan
[7] Graduate Institute of Cancer Biology and Center for Molecular Medicine, China Medical University, Taichung, Taiwan
[8] Department of Biotechnology, Asia University, Taichung, Taiwan

Corresponding author: Dr. Ruey-Hwang Chou and Dr. Yung-Luen Yu, 9F, No.6, Hsueh-Shih Road, Taichung 404, Taiwan. E-mail: rhchou@mail.cmu.edu.tw (Chou, RH), and ylyu@mail.cmu.edu.tw (Yu, YL)
* These authors contributed equally to this work.

Abstract
Post-translational modifications (PTMs) on histones including acetylation, methylation, phosphorylation, citrullination, ubiquitination, ADP ribosylation, and sumoylation, play important roles in different biological events including chromatin dynamics, DNA replication, and transcriptional regulation. Aberrant histones PTMs leads to abnormal gene expression and uncontrolled cell proliferation, followed by development of cancers. Therefore, targeting the enzymes required for specific histone PTMs holds a lot of potential for cancer treatment. In this review article, we retrospect the latest studies in the regulations of acetylation, methylation, and phosphorylation of histones. We also summarize inhibitors/drugs that target these modifications for cancer treatment.

Key words: Histone, post-translational modification, acetylation, methylation, phosphorylation, cancer.

Introduction

The term "epigenome" has been attracting a lot of attention recently. It represents various heritable post-translational modifications (PTMs) on histone proteins or DNA without altering DNA coding sequence (1). These modifications transiently or permanently respond to internal or external stimuli to provide developmental cues. A well-known PTM on DNA is methylation while histones have been reported to undergo methylation, acetylation, phosphorylation, citrullination, ubiquitination, ADP ribosylation and sumoylation (2). Different cues integrate together to direct stem cells to differentiate into various cell types. Dysregulation of this process in development can dictate defects that eventually lead to the susceptibility to diseases. Therefore, PTMs are potential pharmacological targets in many diseases including cancers (3,4). DNA methylation and the other PTMs on histones are major epigenetic features of chromatin by altering nucleosomal organization, and result in gene activation or repression (5). In this review article, we focus on the latest studies in the regulations of acetylation, methylation, and phosphorylation of histones, and also summarize inhibitors/drugs that target these modifications for cancer treatment.

Acetylation/de-acetylation of histones in cancers

Currently, one of the most extensively studied PTMs is histone acetylation (2,6), which adds an acetyl group to the terminal of lysine residues within the tail domain of the core histones. This neutralizes the positive charge of the histones, and results in the relaxation of the chromatin. The relaxed chromatins are more accessible to the transcriptional machinery (6). In particular, acetylated histones provide a binding platform for bromodomain containing proteins to regulate transcriptional activity. Thus, histone acetylation facilitates the maintenance of euchromatin structure and controls transcriptional activation (7). In contrast, histone deacetylation removes acetyl groups from the acetylated histones and leads to an inactive chromatin environment. In general, histone acetyl transferases (HATs) account for the function of transcription co-activators, whereas histone deacetylases (HDACs) act as transcriptional co-repressors (2). The dysfunction of HATs and the increase of HDAC activity are suggested as epigenetic abnormalities in cancers. It has been shown that the dysfunction of tumor suppressor genes is a critical step for carcinogenesis and cancer progression. Notably, epigenetic modulators, such as HDACs, have been suggested to play important roles to suppress the expression of tumor suppressor genes. In addition, through the use of the high-affinity acetyl-lysine antibodies pull down method that couples to the mass spectrometry (MS) detection techniques, almost 2000 acetylated proteins that comprise around 3,600 acetylation sites was identified in the cell (8). The term "acetylome" is therefore created to describe all acetylated sites on a set of acetylated proteins in a cell. These acetylated proteins not only include histone proteins, but also many transcription factors (9). The acetylation status in the cells is tightly regulated by the activities of HATs and HDACs. In normal physiological conditions, the acetylation of histones and transcription factors are precisely regulated. Such homeostasis is regulated by protein activity, concentration, and the re-

cruitment of HATs and HDACs and is essential for gene regulation of normal cellular functions. In addition, it has been shown that differential recruitment of HATs and HDACs also regulates the acetylation homeostasis, and has been linked to various neurodegenerative disorders and cancerous diseases. In the process of tumor formation, the acetylation machinery is impaired and the acetylation status in cells becomes deacetylation, thus resulting in dysregulation of proliferation, differentiation and apoptosis and, further transforming the cells into a malignant state and the initiation and progression of cancer (10).

The enzymes, HATs and HDACs, regulate the acetylation of histones to modulate gene expression. It has been shown that the balance between HATs and HDACs is dysregulated in many types of cancer. Currently, HDACs are classified into four groups namely class I (HDACs 1-3, and HDAC8), class II (HDACs 4-7, HDACs 9-10), class III (sirtuins) and class IV (HDAC11). HDAC inhibitors have been demonstrated to exert anticancer activity by promoting acetylation of both histones and non-histone protein substrates. Administration of HDAC inhibitors to cancerous cells results in cell cycle arrest, DNA repairs inhibition, and the induction of cell apoptosis.

Tumor suppressor genes negatively regulate the initiation and progression of cancers. HDACs, the histone acetylation erasers, have been shown to silence the tumor suppressor genes. During the initiation phase of cancer, HDACs play roles in silencing the expression of genes that control cell cycle and differentiation. The elevated HDAC activity has been observed in promyelocytic leukemia (11), non-Hodgkin lymphoma (12), colon cancer and gastric cancer (13).

HDACs and cancer cell proliferation

Dysregulation of genes that negatively regulate cell proliferation is a hallmark of carcinogenesis. HDACs participate in the regulation of cell proliferation through repressing the expression of selective inhibitors, such as p21 and p27, of cyclin dependent kinase (CDK). Notably, HDAC1-null mouse embryos and ES cells showed increased levels of p21 and p27 that are correlated with a decreased level of cell proliferation (14). It has been demonstrated that HDACs cooperate with SP1 to repress p21 and enhances proliferation of mouse embryonic fibroblasts (14,15). In addition, HDAC4 also cooperates with Sp1/Sp3 to repress p21 expression in colon cancer cells (16) and in glioblastoma cells (17). Moreover, silenced HDAC4 upregulates the expression of p21 and thus inhibits human glioblastoma cell growth *in vitro* and *in vivo* (17). Furthermore, the expression levels of HDACs, such as HDAC1 (18-20), HDAC2 and HDAC3 (21) are highly upregulated in prostate cancer.

HDACs and cancer cell differentiation

Dysregulated cancer cell proliferation is usually accompanied by the loss of differentiation capacity. HDACs have been demonstrated to play important roles in the regulation of histone acetylation, thus affecting the expression of differentiation-related genes. In addition, Mucins are a family of proteins that are highly glycosylated. Of note, Mucin2 (MUC2) is the most abundant gastrointestinal secreted form that is involved in gastrointestinal cell differentiation. Decreased MUC2 expression is observed in both pancreatic and colorectal cancers. MUC2-null mice have been demonstrated to develop adenocarcinoma (22). In particular, H3-K9 and H3-K27 acetylation has been reported to be enhanced in MUC2 promoter region to activate the expression of MUC2. Trichostatin A (TSA), a HDAC inhibitor, has been shown to induce MUC2 mRNA and protein expression in the pancreatic cancer cells that lack MUC2 expression (23). Furthermore, treatment of colon cancer cells with sodium butyrate, another HDAC inhibitor, has also been demonstrated to induce MUC2 mRNA and protein expression through enhancing the acetylation of H3 (24). In addition, the increased acetylation of histone H3 and H4 at the MUC2 promoter and upregulation of MUC2 mRNA were also observed upon sodium butyrate treatment (25).

HDACs and the regulation of cancer cell apoptosis

In addition to cell proliferation and differentiation, HDACs also play a role to suppress apoptosis in cancer cells. For example, HDAC2 reduce apoptosis of pancreatic cancer cells (26). Furthermore, knockdown of HDAC2 in pancreatic cancer cells accelerates the processing of caspase 8 and increases apoptosis. Additionally, co-treatment of valproic acid (VPA) in HDAC2 suppressing pancreatic cells further enhances apoptosis of them (26). Interestingly, knockdown of HDAC2 upregulates the expression of NOXA gene, which is pro-apoptotic and can sensitize pancreatic cancer cells to etoposide-induced apoptosis, suggesting that HDAC2 can silence NOXA in pancreatic cancer cells (27). Similarly, knockdown of HDAC1 also enhances apoptosis in osteosarcoma cells (28), whereas knockdown of HDAC2 induces apoptosis in HeLa cells (12). In particular, synergistic treatment of two HDAC inhibitors, VPA and CI-994, can lead to the stabilization of histone acetylation and the restoration of caspase-8 in small cell lung carcinomas cells (29). Notably, treatment of medulloblastoma cells with HDAC inhibitor, MS-275, induces caspase-8 activity and increases apoptosis. This coincides with the increased acetylation levels of histone H3 and H4 at the promoter of TRAIL receptor 1 and increased levels of TRAIL receptor 1 gene and protein (30). In lung cancer cells, silence of HDAC2 induces cellular apoptosis through the activation of p53 and Bax activation and the suppression of Bcl2. In contrast, overexpression of HDAC2 inhibits p53 expression and Bax, as well as increases the expression of Bcl2, thus let the transformed cells overcoming the apoptotic signal (31). Moreover, treatment with another HDAC inhibitor, suberanilohydroxamic acid (SAHA; also named as vorinostat), also sensitizes breast cancer cells and induce the activation of caspase 8, caspase 3, Bid and PARP cleavage concomitantly, as well as the enhanced expression of Bax and TRAIL receptor 1 (32). *In vivo*, treatment of the breast cancer cells in nude mice with SAHA induce the expression of Bax, Bim, NOXA, p21, DR4, DR5, tissue inhibitor of metalloproteinase-1 (TIMP-1) and TIMP-2 (33). HDAC5 is required for the maintenance of heterochromatin and the refractory

to apoptosis of human cancer cells. In contrast, loss of HDAC5 induced heterochromatin de-condensation and enhanced sensitivity of DNA mutation agents, thus resulting in cancer cell apoptosis (34). Currently, it is clear that HDAC1, HDAC2 and HDAC5 are involved in the attenuated apoptosis during carcinogenesis.

Cancer progression and the roles of HDACs

In addition to the initiation of cancer, HDACs also regulate the genes implicated in the progression of cancer. Decreased histone acetylation has been suggested to be involved in tumorigenesis, tumor invasion and metastasis (35). Furthermore, the status of acetylation and deacetylation of the transcription factor HIF1α regulate angiogenesis and cellular metabolism in carcinogenesis. The acetylation and deacetylation at different lysine residues in HIF1α protein result in different biological effects. Acetylation at the N-terminus (Lys-10, 11, 12, 19, 21) and at the oxygen-dependent degradation domain (Lys-532) can promote HIF1α protein degradation and inhibition of downstream HIF1 activity (36). In particular, HDAC4 can deacetylate the N-terminal of HIF1α and increases the stability, transcriptional activity of HIF1α and thus the expression of a subset of HIF1α downstream target genes, including VEGF-α, lactate dehydrogenase A, and GLUT1 (36). Furthermore, such deacetylation directs the cells towards transformation. Notably, glycolytic genes, such as phosphoglycerate kinase 1 and pyruvate kinase M2 and glucose transporters GLUT1 and GLUT3, are also the downstream targets of HIF1α and are upregulated in cancer cells (37-39). Therefore, HDACs regulate the metabolic changes in cancer cells through the deacetylation machinery to meet the demand of extra energy requirement of cancer cells.

HDAC inhibitors

HDACs have attracted a lot of attention as anticancer therapeutic targets. It has been suggested that HDACs are involved in tumorigenesis (40,41). HDACs negatively regulate the gene expression of cell cycle inhibitors, differentiation factors, and pro-apoptotic factors. Notably, HDACs also up-regulate the expression of genes associated with angiogenesis, cell invasion and migration. In the previous sections, we have shown that HDACs are involved in the regulation of cancer initiation and progression. Therefore, HDAC inhibition may show promising antitumor effects on various types of cancer. Specifically, HDAC inhibitors could exert their anti-cancer effects through inducing cell cycle arrest, apoptosis, and differentiation (40,41). In addition, the specific HDAC inhibitors should be designed to avoid in targeting all of the HDACs. Due to different chemical structures and mechanisms of inhibition, seven categories of HDAC inhibitors have been reported, including short chain fatty acids, cyclic peptides, benzamides, hydroxamine-acid-derived compounds, electrophilic ketones, miscellaneous compounds and sirtuin inhibitors. Inhibitors for class I, II, IV of HDACs have a common metal binding domain that functions to block Zn^{2+} chelation at the active site (42). In particular, zinc-dependent HDAC inhibitors are ineffective against class III HDACs (sirtuin) (43). The mechanisms of action and the biological consequences of Class III HDAC inhibitors remain unclear (44-46). Furthermore, sirtuin inhibitors were shown to be ineffective in human cells (47). Nevertheless, specific inhibitors against sirtuin I (SEN96) (48) and sirtuin 2 (compound 6J) (49) are currently in clinical trials. Zinc-dependent HDAC inhibitors have been developed as anticancer drugs, and two sirtuin inhibitors have been approved for cancer treatment (50). Specifically, inhibition of HDAC results in cell cycle arrest at G1 phase and upregulation of p21 in a p53-independent manner (51) through increase of histones H3 and H4 acetylation at the p21 promoter (52). In addition, HDAC inhibitors, such as butyrate and TSA, exert the anti-cancer effects by stabilizing p21 mRNA (52), repressing cyclins A, D, and activating p16 and p27 in cell cycle arrest (53,54). Interestingly, TSA has also been reported to induce microRNA-7 expression, leading to suppression of epidermal growth factor receptor (EGFR) expression in an HDAC-independent manner in lapatinib-treated breast cancer cells (55). Furthermore, treatments of HDAC inhibitors have been shown to activate the expression of pro-apoptotic genes and/or to reduce the expression of anti-apoptotic genes (56). Comparing with other epigenetic drugs, the potency is the major advantage of HDAC inhibitors, due to that most of their effective doses are in nano- and micro-molar range (57,58). It has also been shown that HDAC inhibitors also inhibit angiogenesis and increase the host immune system in cancer patients (56,59,60). Therefore, HDAC inhibitors can be used with other different drugs in cancer therapies. Notably, HDAC inhibitors can function synergistically with different chemotherapeutic agents and biologic polypeptides. For example, pretreatment with the HDAC inhibitor, vorinostat, enhances the drug effects of topoisomerase II inhibitors (61). In addition, HDAC inhibitors are also combined with DNA demethylating agents to reactivate the silenced tumor suppression genes. As a proof of principle, decitabine was combined with HDAC inhibitors (such as phenyl butyrate or VPA) to treat the patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS). The results have been shown more tolerable and promising efficacy in three Phase I/II trials (62-64). Notably, another promising strategy is the use of HDAC inhibitors with tyrosine kinase inhibitors in cancers that are characterized with overexpressed anti-apoptotic genes. For example, vorinostat, LBH-589, LAQ-824 and romidepsin have been demonstrated there synergistic effects with imatinib for pro-apoptotic activity in the treatment of both imatinib-sensitive and imatinib-resistant leukemic cells (65-67).

HAT inhibitors

HAT is responsible for the reaction of histone acetylation, which transfers the acetyl group of acetyl coenzyme A (acetyl-CoA) to the ε-amino group of lysine (68,69). HATs have been classified into two groups, type-A and B, based on sequence divergence of the HAT domain and intracellular localization. Type A HATs are nuclear localized HATs that acetylate histones and other chromatin-associated proteins. Interestingly, although Type B HATs are localized in both nuclear and cytoplasm, it is responsible that acetylating the newly

synthesized histones in the cytoplasm promotes their nuclear localization (70,71). Type A HATs consist of three families: GNATs, P300/CBP, and MYST. Type B HAT consist only the histone acetyltransferase-1 (HAT1/KAT1) (69,72). Little sequence similarity and no homology domain have been shown in the type A HAT families, but the acetyl-CoA binding domain is highly conserved in all HATs (73).

Due to the specific catalytic activity of HATs relying on association, HAT inhibitors are therefore mainly the acetyl-CoA-related derivatives, conjugates. Currently, HAT inhibitors are classified into synthetic peptide CoA-based, natural product or small molecule. Notably, the synthetic HAT inhibitors were identified from the finding that polyamine-CoA conjugates can inhibit HAT activity *in vitro* (74). Most of the synthetic bi-substrate HAT inhibitors mimic the complex of acetyl CoA-lysine intermediate and exert the inhibitory effects. The major disadvantage for this class of HAT inhibitors is their high degree of cellular impermeability. Furthermore, most of the HAT inhibitors from natural products also have the same disadvantage. The best characterized of HAT inhibitors from natural products is Curcumin, which is isolated from the rhizome. It has been demonstrated that Curcumin is effective in the prevention and treatment of many types of cancer, such as colorectal, breast, cervical kidney, lung, prostate, ovarian and liver cancers (75,76).

The third class of HAT inhibitors is a number of small molecules to deal with permeability. These inhibitors include butyrolactone 3 (MB-3), quinoline and isothiazolone and their derivatives. Isothiazolone has been shown to inhibit the cell proliferation of human ovarian and colon cancer cell lines (77). The derivatives of isothiazolone also showed inhibitory effects on the enzyme activities of HAT. Using HATs as drug targets is complicated due to the fact that most HATs are the component of large protein complexes (78), which may increase the variability of HATs. It has been shown that the protein complexes have been shown to reduce the HAT activity from certain loci (79). In addition, the protein complexes facilitate the different enzymatic activity required for different cellular functions. Based on these findings, making HAT inhibitors with cell permeability will facilitate the drug development for cancer treatment.

Methylation/de-methylation of histones in cancers

The dynamical activities of lysine methyltransferases (KMTs) and demethylases (KDMs) control the methylation status of lysine residues on histone proteins, which modulate chromatin structure to regulate different cellular functions, including transcription, replication and repair (80). The specificity and degree of methylation at lysine residues rely on particular KMTs and KDMs. The first site-specific histone KMT, SUV39H1 (KMT1A), which contains a conserved enzymatic SET domain, has been identified in 2000 (81).The SET domain, a 130 amino acid with catalytic activity towards lysine residues of histones, was initially found to be conserved in Su(var)3-9, E(z) (enhancer of zeste) and trithorax (82). By homology alignments with the SET domain, dozens of KMTs have further identified (83). In addition to SET domain-containing KMTs, another class of KMTs without SET domain, such as KMT4 (also known as Dot1p in yeast and Dot1L in human), has also been clarified (84,85). Although these two classes of KMTs do not possess conserved catalytic domains, both of them utilize S-adenosyl-L-methionine (SAM) as the methyl group donor for lysine methylation on histones (83,86). The presence of histone KDMs was first identified from a part of the C-terminal binding protein 1 (CtBP1) corepressor complex as LSD1/KDM1A, which contains a flavin adenine dinucleotide (FAD)-dependent amine oxidase domain that demethylates H3K4me2 and H3K4me1 and modulates gene expression (87,88). Additionally, another type of histone KDMs, such as KDM2A/B, which utilizes the Jumonji (JmjC) domain to catalyze demethylation through the oxidation of methyl groups, is dependent on a-ketoglutarate, molecular oxygen, and Fe(II) (89-91). However, the aforementioned KDM1 and KDM2, as well as KDM3 can't demethylate trimethylated lysine until the discovery of KDM4A-KDM4D (also known as JMJD2A-JMJD2D), which are capable of demethylating histone H3K9me3/H3K9me2, H3K36me3/H3K36me2, and H1.4K26me3/H1.4K26me2, but are unable to remove H3K9me1 or H3K36me1 (92-96). These studies demonstrate the specificity of KMTs and KDMs for both the site and degree of methylation.

The site and the degree of methylation on lysine residues are important in generation of open or closed chromatin. For instance, histones H3K4me3, H3K36me2/3, H3K79me2/3, and H4K20me1 correlate with active states of open chromatin; in contrast, H3K9me3, H3K27me3, and H4K20me3 marks are associated with closed heterochromatin (97,98). The above histone 'marks' provide a particular surface for recognition by 'reader' proteins. For example, the basal transcription factor TFIID directly recognizes and binds to the H3K4me3 mark via the plant homeodomain (PHD) finger of TAF3, leading to activation of transcription (99). In contrast, a heterochromatic adaptor HP1 recognizes H3K9me3 mark through its chromo domain, thereby generating the closed supra-nucleosomal chromatin structure and silencing transcription (100). Accumulated evidences demonstrate that lysine methylation of histones play important roles in cancer progression. Alterations of histone KMTs and KDMs, such as overexpression, downregulation, mistargeting, mutations and gene fusions caused by chromosomal translocations, result in aberrant modification marks and structures of chromatin, leading to deregulation of cancer-specific gene expressions, uncontrolled cell proliferation, and genome instability, in different types of cancers (101). For example, KMT5A (also known as PR-SET7 and SET8) directly interacts with PCNA and mono-methylates histone H4K20 to regulate S phase progression during the cell cycle (102,103). The amounts of KMT5A and histone H4K20me1 increase with cell cycle progression, reach to the highest levels during G2/M and early G1 phase (104), subsequently, followed by marked degradation of KMT5A via PCNA-coupled CRL4 (CDT2) ubiquitination during S-phase (105). KMT5A and H4K20 methylation are tightly regulated in replication fork to prevent aberrant re-replication and maintain fork stability during DNA replication (106,107). His-

tone H4K20me1 can be further di- and tri-methylated by Suv4-20h1/2(107). Histone H4K20me2 is required for recruiting 53BP1 at sites of DNA damage to initiate DNA repair process (108). We also find a crosstalk of histone H4K20 methylation and H4Y72 phosphorylation in regulation of the above processes (109). Histone H4K20me3 and Suv4-20h enzyme coupled with HP1 are focally enriched at pericentric heterochromatin in higher order chromatin structure (110). In addition, KMT5A interacts with TWIST, a master regulator of epithelial–mesenchymal transition (EMT), to activate the transcription of N-cadherin gene and repress that of E-cadherin gene via its mono-methylation activity towards H4K20, thereby promoting EMT and enhancing the invasive potential of breast cancer cells both *in vitro* and *in vivo*. Importantly, the expression of KMT5A is clinically positive-correlated with expressions of N-cadherin and TWIST expression, as well as metastasis, but negative-correlated with E-cadherin expression in human breast carcinoma, suggesting KMT5A as a potential therapeutic target of breast cancer via suppressing metastatic properties (111).

DOT1L (as known as KMT4) specifically methylates histone H3K79 at various degree including mono-, di- and tri-methylation (85,112). Although DOT1L does not possess a common SET domain, its catalytic domain shows structural similarity with that of classic non-histone methyltransferases (113), such as catechol O-methyltransferase COMPT (114). Mistargeting of DOT1L by association with different MLL fusion proteins, such as MLL-AF4, MLL-AF9, MLL-AF10, and MLL-ENL caused by translocation, leading to their abnormal expressions have been demonstrated in leukemias (115,116). Additionally, the activity of DOT1L is required for maintaining the MLL-AF6-driven oncogenic gene-expression program in accordance with high levels of histone H3K79me2 in hematologic malignancy. Conditional knockout of Dot1l results in suppressing the MLL-AF6-mediated leukemogenesis in a mouse model (117).

Polycomb group (PcG) proteins serve as the regulators to control the process through histone modifications (118,119). PcG proteins comprise of two polycomb repressor complexes (PRCs), PRC1 and PRC2. The PRC2 complex consists of EZH2, SUZ12, EED, and RBAP48, in which EZH2 is a catalytic subunit with histone methyltransferase activity that tri-methylates lysine 27 of histone H3 (H3K27me3). Previous studies have demonstrated that EZH2 is overexpressed in a wide variety of cancerous tissue types, such as prostate, breast (119-121), and brain (122-125) cancers. Its roles in maintenance of stemness and neuron regeneration have been illustrated (126-129). In addition, accumulating evidences have demonstrated that EZH2 is critical in drug resistance and overexpressed in cancer stem cells (also named as tumor initiating cells, TICs). For instance, high expression of EZH2 is associated with tumor aggressiveness and poor prognosis in patients with esophageal squamous cell carcinoma treated with definitive chemoradiotherapy (130). Overexpression of EZH2 contributes to the acquired cisplatin resistance in ovarian cancer cells (131). Overexpression of EZH2 and chemotherapy have been shown to enrich stem cell-like side populations in ovarian cancer (132), breast cancer (133), and prostate cancer (134). In contrast, decreased expression of EZH2 shows favorable outcome to tamoxifen, an antagonist of the estrogen receptor in advanced breast cancer (135,136). Knockdown of EZH2 by RNAi re-sensitizes drug-resistant ovarian cancer cells to cisplatin (131), reverses the drug resistance to 5-Fu in human hepatic multidrug-resistant cancer cells (137), and decreases MDR1 expression and sensitizes multidrug-resistant hepatocellular carcinoma cells to chemotherapy (138).

Histone KMT inhibitors

Due to the roles of KMTs/KDMs in cancers, several inhibitors against some of these molecules have been developed. The aminonucleoside inhibitors, such as EPZ004777, have been used for targeting DOT1L catalytic activity by competition with SAM for binding to its active site. The compound EPZ004777 selectively decreases the global levels of H3K79 methylation and kills mixed lineage leukemia cells bearing the MLL gene translocation(139,140). Although it has been reported that activation of Wnt-targeted genes also depends on H3K79 methylation, recently, treating human colon adenocarcinoma-derived cell lines by inhibiting DOT1L with EPZ004777 does not affect the canonical Wnt signaling pathway and H3K79 methylation is not increased in clinical human colon carcinoma samples comparing with that in normal colon tissue, suggesting that DOT1L might be not a favorable therapeutic target in colon cancer (141). Another advanced DOT1L inhibitor, EPZ-5676, has been designed and synthesized as a SAM-competitor, which induces conformational changes in the active site, and possesses attractive selectivity and a slow off-rate with the most potent effects on inhibition of H3K79 methylation and MLL-fusion target gene expression, leading to effective cell killing for acute leukemia (142). It demonstrates biexponential pharmacokinetics following intravenous administration, but has low oral bioavailability in animal models including mouse, rat and dog (143). EPZ-5676 is also effective in AML with partial tandem duplication (PTD), MLL-PTD (144). Moreover, the synergistic anti-proliferative activity of EPZ-5676 has been observed in combination with cytarabine and daunorubicinin MLL-rearranged leukemia cells (145). Recently, EPZ-5676 has been applied to a phase I clinical trial in the adult and pediatric patients with MLL-rearranged AML (http://clinicaltrials.gov).

A series of small molecules, such as GSK126 (146), EI1 (147), UNC-1999 (148), and E7438 (EPZ-6438) (149), have been developed for inhibition of the catalytic activity of EZH2 and thereby decreasing global tri-methylation at histone H3K27. These inhibitors are selectively against EZH2 over other KMTs and function by competition with SAM. The turnover rates of H3K27me3 are low, thus prolong treatment of EZH2 inhibitors is required for its efficacy in non-Hodgkin's lymphoma without altering H3K27me1 (150). EZH2 is normally expressed in the germinal center (GC) B cells and decreases to turn on genes required for B cells differentiation. Around 1800 EZH2-targeted genes in GC B cells are identified. Accordingly, knockdown of EZH2 in diffuse large B-cell lymphoma (DLBCL) cells leads

to acute cell cycle arrest at the G1/S transition and up-regulation of its tumor suppressor target genes. These results suggest the critical roles of EZH2 involved in regulation of a specific epigenetic program in normal GC B cells, and aberration of the epigenetic program may contribute to their malignant transformation into DLBCLs (151). Somatic mutations of EZH2 frequently occur in B cell lymphomas. Conditional expression of mutant EZH2 in mice promotes hyperplasia and lymphomagenesis via abnormal suppression of genes required in B-cell differentiation. The discovery provides the potential therapeutic strategy by targeting EZH2 in DLBCLs (152). EZH2 inhibitors have effective anti-tumor activity in different animal models including GCB-DLBCL xenograft models (146,149,150,152) and SMARCB1-deletion driven rhabdoid tumor models (153). The clinical trials of EZH2 inhibitors, including E7438 (EPZ-6438), CPI-1205, and GSK2816126, are in progress for different types of lymphomas (http://clinicaltrials.gov). The anti-tumors activity of other histone KMT inhibitors, such as A-366 against EHMT1 (154), BIX-01294 against EHMT2 (155-157), AZ505 and LLY-507 against SMYD2 (158,159), have also documented in preclinical stage.

Histone KDM inhibitors

Human KDMs for histones consist of two distinct enzyme classes: FAD-dependent amine oxidase type demethylase, such as LSD1/KDM1A (87,88), and the JmjC-domain containing demethylases, such as KDM2A/B (89-91). Tranylcypromine (TCP) is an irreversible inhibitor of KDM1A via covalently binding to the FAD co-factor that resides at the base of the active site (160). The cellular effects of the KDM1A inhibitors on induction of histone H3K4 methylation and anti-proliferative activity are demonstrated as well (161). Afterward, a variety of TCP analogs have been generated by substituting the phenyl group of TCP with functional groups ranging from fluoro- and bromo-additions (162,163). TCP analogs, trans-N-[1-(2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]-2-phenylcyclopropan-1-amine (Compound A) and trans-N-[(2-methoxypyridin-3-yl) methyl]-2-phenylcyclopropan-1-amine (Compound B, also known as Oryzon), effectively inhibit KDM1A-mediated responses in the nanomolar range and show similar phenotypes of Kdm1a knockdown in both murine and primary human AML cells bearing the MLL gene translocations exhibiting MLL translocations(164). Two irreversible, TCP-derived KDM1A inhibitors, ORY-1001 (165) and GSK2879552 (165,166), are currently being applied to clinical trials in patients with AML or small cell lung cancer (http://clinicaltrials.gov).

Another type of KDM1A inhibitors is reversible, such as GSK690 and N'-(1-phenylethylidene)-benzohydrazides (167,168). The most potent inhibitor, SP2509, attenuates the interaction between LSD1 and the corepressor CoREST, enhances the permissive H3K4me3 mark on the target gene promoters, and increases the levels of p21, p27 and CCAAT/enhancer binding protein alpha, leading to suppressing cell growth in AML cells. Combinational treatment with SP2509 and panobinostat, a pan-HDAC inhibitor, synergistically kills AML cells and also improves the survival in a human AML cell-inoculated mouse model (169). In addition, low molecular weight amidoximes, such as bis-guanidines, bis-biguanides, and their urea- and thiourea isosteres, have been identified as KDM1A inhibitors by structure-based virtual screening. These amidoximes potently inhibit LSD1/ KDM1A and induce the re-expression of aberrantly silenced tumor suppressor genes in tumor cells (170). However, the potentials of these novel reversible KDM1A inhibitors in pre-clinical development need to be further explored and evaluated.

The other KDM members containing the JmjC domain utilize 2-oxoglutarate (2-OG; α-ketoglutarate) as a co-factor. Several inhibitors of JmjC domain-containing KDMs have been designed by interfering with the association between KDMs and 2-OG (171). For instance, GSK-J1 is the first selective and potent H3K27-specific demethylase inhibitor from high throughput screening. GSK-J1 mimics 2-OG binding by a propanoic acid moiety, and also chelates the Fe^{2+} on active site by a pyridyl-pyrimidine biaryl, thereby inducing conformational change of this catalytic divalent cation in the active site to suppress the activity of KDMs. A cell-permeable derivative of GSK-J1 is synthesized by esterification of its polar carboxylate group and named as GSK-J4 (172). The effects of GSK-J4 on tumor suppression have been observed in T-ALL leukemia cells (173).

Phosphorylation of histones in cancers

Mitogen- and stress-activated kinase 1/2 (MSK1/2) elicits histone H3 phosphorylation at S10 (H3S10p) and S28 (H3S28p) (174), in which H3S10p contributes to EGF-stimulated neoplastic cell transformation (175). The MAP kinase cascades are key regulators in the phosphorylation of H3S10 and activation of immediate early (IE) response genes upon different stimuli, including growth factors, cytokines, and stress. Increased H3S10p induces the expression of the IE response genes, including proto-oncogenes c-fos and c-jun, in cancer progression (176). Activating mutations in K-Ras elevates the Ras-MAPK pathway and results in the MSK1-mediated H3S10p in neoplastic transformation of pancreatic cancer cells (177). The transcription byproducts, R loops, constitute a threat to genome integrity and are tightly linked to H3S10p for chromatin condensation (178,179). In addition to acetylation and methylation of histones, gene transcription is also regulated through phosphorylation of histone H2B at S33 by TAF1 (180) and histone H3 at T11 by PRK1 (181). Moreover, tyrosine phosphorylation of histones has also been identified in recent years. WSTF, a transcription factor, has intrinsic tyrosine kinase activity toward Y142 of histone H2AX to maintain S139 phosphorylation and IR-induced foci formation, which is crucial for regulation of the DNA damage response (182). Rad53-associated Y99 phosphorylation of histone H3 is also critical for efficient ubiquitination and degradation in the regulation of histone levels (183). JAK2 phosphorylates histone H3-Y41 and prevents HP1α binding to chromatin (184). WEE1 has been identified as the tyrosine kinase towards Y37 on histone H2B, and histone H2B-Y37 phosphorylation suppresses expression of replication-dependent core histone genes via excluding binding of the transcriptional coactivator NPAT and RNA poly-

merase II, and recruiting the histone chaperone HIRA (185). Recently, we have demonstrated that EGFR can translocate into the nucleus to phosphorylate histone H4 at Y72 and results in enhancing the recruitment of histone KMTs to facilitating its K20 methylation and consequently promotes DNA synthesis and repair (109). The evidence supports that non-canonical nuclear EGFR works as a modifier of nuclear proteins, such as PCNA (186), RNA helicase A (187), PNPase (188), and histone H4 (109), and thus regulates the relative cellular functions. Disruption of the interaction between EGFR and the associated nuclear proteins leads to suppress cell growth of prostate cancer (189) and breast cancer (109,190).

Kinase inhibitors

Based on the previous documents, several kinases have been known to possess kinase activity toward histones, such as MSK1 (174,177), JAK2 (184), WEE1 (185), and EGFR (109). Inhibition of MSK1 activity by addition of its inhibitor, H89, or by knockdown of MSK1 with specific short hairpin RNAs (shRNAs) specifically abrogates cell proliferation in response to estrogens or progestins in breast cancer cells (191). Recently, to Inhibition of MSK1 by the same approach significantly reduces latent membrane protein 1 (LMP1)-promoted cell proliferation, and induces cell cycle arrest at G0/G1 phase. Knockdown of MSK1 attenuates the LMP1-promoted anchorage-independent cell growth in nasopharyngeal carcinoma cells (192). Because of the off-target effects of MSK inhibitor H89, another more specific MSK1 inhibitor, SB-747651A, has been developed with an IC50 value of 11 nM. Cellular study reveals that SB-747651A inhibits production of the anti-inflammatory cytokine IL-10 (193). Treatment with SB-747651A dramatically diminishes invasiveness of aggressive oral squamous cell carcinoma (194).

Somatic mutations of JAK2 such as JAK2-V617F, a recurring gain-of-function mutation, are correlative to development of myeloproliferative disorders (MPD). Small-molecule inhibitors of JAK2 kinase can be classified into two types: JAK2-selective (Class I) and non-JAK2 selective (Class II). The JAK2-selective inhibitors, including INCB018424, XL019, and G101348, and non-JAK2 selective inhibitors, such as MK-0457 (VX680), CEP-701 (Lestaurtinib), and AT9283, have been employed in clinical trials in patients with MPD, acute lymphoblastic leukemia (ALL), and CML (195). The type II inhibitor of JAK2, CHZ868, stabilizes JAK2 protein in an inactive conformation. CHZ868 potently inhibits the growth of CRLF2-rearranged human B-ALL cells, disrupts JAK2 signaling, and improves survival in B-ALL cells-inoculated mice. Combinational treatment with CHZ868 and dexamethasone synergistically induces apoptotic response in JAK2-dependent B-ALLs comparing to CHZ868 alone (196). These findings support the therapeutic strategy for the patients with JAK2-dependent leukemias and other disorders by targeting JAK2 kinase.

WEE1 kinase is a key G2-M checkpoint regulator, which phosphorylates CDC2 at Y15, leading to inactivation of the CDC2/cyclin B complex and induction of cell cycle arrest at G2/M. Inhibition of WEE1 by either small molecule inhibitors or shRNAs results in premature entry into mitosis and consequently inducing cell death via mitotic catastrophe or apoptosis (197). A series of WEE1 inhibitors have been synthesized, including PD0166285 (198,199) and MK-1775 (also named as AZD1775) (200-208). In addition to the small compound inhibitors, a microRNA, miR-381, has been identified as a novel intrinsic WEE1 inhibitor, which up-regulates CDC2 activity to sensitize renal cancer cells to 5-FU by induction of mitotic catastrophe and apoptosis (209). MK-1775 (AZD1775) has been employed in clinical trials in patients with wide types of cancers including lung cancer, ovarian cancer, fallopian tube cancer, peritoneal cancer, cervical cancer, glioblastoma, pancreatic cancer, head and neck cancer, and AML (http://clinicaltrials.gov).

Overexpression of EGFR have been observed in different types of cancers, including ovarian cancer (210), breast cancer (211-215), lung cancer (216-219), colorectal cancer (220). Several tyrosine kinase inhibitors (TKIs) against EGFR are currently used in clinical treatment of cancers, such as Erlotinib (Tarceva) (221), Gefitinib (Iressa) (222), Erbitux (Cetuximab) (223) and Tykerb (Lapatinib), which is a dual TKI to EGFR and HER2 (224). Lapatinib promotes the sensitivity to proteasome inhibitors via inducing NF-kappaB activation in triple-negative breast cancer cells (225). However, drug resistance to these clinical used TKIs sometimes occurs. The worse event-free survival rate of lapatinib in breast cancer may partly attribute to the elevation of EGFR through the downregulation of microRNA-7, subsequently leading to overexpression of cyclooxygenase-2 independent of EGFR kinase activity (226). Moreover, the specific monoclonal antibody against EGFR, such as Panitumumab, has also been developed for target therapy in cancer (227).

Conclusion

We described the machinery to maintain the homeostasis of PTMs on histones by specific enzymes, such as HATs/HDACs, KMTs/KDMs, and kinases, within cells. Abnormal activation of these enzymes frequently occurs in different types of cancers. Several inhibitors/drugs have been developed for specifically suppressing the overexpressed enzymatic activity in pre-clinical or clinical treatment of cancers (summarized in Table 1). Targets validation for personalized medication, and the specificity, biostability, and reactivity of the inhibitors/drugs are critical for the outcome of cancer patients after treatments. Importantly, drug resistance is another difficult issue needed to be solved. Compensation with activating alternative signal pathways has been uncovered in cells with drug resistance. Therefore, combinational treatments with different inhibitors/drugs might overcome the difficult problems. To sum up, understanding the detail molecular mechanisms of specific inhibitors/drugs and their resistance will benefit to improve the therapeutic strategy for better clinical outcome of cancer patients.

Acknowledgements

We appreciate the financial support by grants from Ministry of Science and Technology (MOST103-

Table 1. The candidate targets and inhibitors of histone PTMs in oncology.

	Targets	Inhibitors
HATs	p300/CBP	Curcumin
	p300/CBP	Quinoline
	PCAF and p300/CBP	Isothiazolone
	GCN5	Butyrolactone 3 (MB-3)
HDACs	pan-HDACs	LBH-589 LAQ-824 Vorinostat (SAHA: suberanilohydroxamic acid) Panobinostat
	class I and II HDACs	Trichostatin A (TSA)
	class I HDAC	Valproic acid (VPA) CI-994
	HDACs 1, 2 and 7	Sodium butyrate
	HDAC1 and HDCA2	romidepsin
	sirtuin I	SEN96
	sirtuin 2	Compound 6J
KMTs	DOT1L/KMT4	EPZ004777 EPZ-5676
	EZH2	GSK126 GSK2816126 EI1 UNC-1999 E7438 (EPZ-6438) CPI-1205
	EHMT1	A-366
	EHMT2	BIX-01294
	SMYD2	AZ505 LLY-507
KDMs	LSD1/KDM1A	Tranylcypromine (TCP) Compound A (trans-N-[1-(2,3-dihydro-1,4-benzodioxin-6-yl)ethyl]-2-phenylcyclopropan-1-amine) Compound B (Oryzon) ORY-1001 GSK2879552 GSK690 N'-(1-phenylethylidene)-benzohydrazides SP2509 Bis-guanidines Bis-biguanides
	JmjC domain-containing KDMs	GSK-J1 GSK-J4
Histone kinases	MSK1	H89 SB-747651A
	JAK2	INCB018424 XL019 G101348 MK-0457 (VX680) CEP-701 (Lestaurtinib) AT9283 CHZ868
	WEE1	PD0166285 MK-1775 (AZD1775)
	EGFR	miR-381 Erlotinib (Tarceva) Gefitinib (Iressa) Erbitux (Cetuximab) Tykerb (Lapatinib) Panitumumab

2320-B-039-052-MY3, MOST104-2321-B-039-005, MOST104-2320-B-039-031, MOST103-2314-B-715-001-MY2, and MOST104-2314-B-715-003-MY3), National Health Research Institutes (NHRI-EX102-10245BI), China Medical University (CMU104-S-02), Taiwan.

Other articles in this theme issue include references (228-239).

References

1. Goldberg, A. D., Allis, C. D., Bernstein, E. Epigenetics: a landscape takes shape. *Cell*, 2007, **128**:635-638, doi:10.1016/j.cell.2007.02.006

2. Parbin, S., Kar, S., Shilpi, A., Sengupta, D., Deb, M., Rath, S. K., et al. Histone deacetylases: a saga of perturbed acetylation homeostasis in cancer. *J Histochem Cytochem*, 2014, **62**:11-33, doi:10.1369/0022155413506582

3. Alhazzazi, T. Y., Kamarajan, P., Verdin, E., Kapila, Y. L. SIRT3 and cancer: tumor promoter or suppressor? *Biochim Biophys Acta*, 2011, **1816**:80-88, doi:10.1016/j.bbcan.2011.04.004

4. Alhazzazi, T. Y., Kamarajan, P., Joo, N., Huang, J. Y., Verdin, E., D'Silva, N. J., et al. Sirtuin-3 (SIRT3), a novel potential therapeutic target for oral cancer. *Cancer*, 2011, **117**:1670-1678, doi:10.1002/cncr.25676

5. Vaissiere, T., Sawan, C., Herceg, Z. Epigenetic interplay between histone modifications and DNA methylation in gene silencing. *Mutat Res*, 2008, **659**:40-48, doi:10.1016/j.mrrev.2008.02.004

6. Grunstein, M. Histone acetylation in chromatin structure and transcription. *Nature*, 1997, **389**:349-352, doi:10.1038/38664

7. Shogren-Knaak, M., Ishii, H., Sun, J. M., Pazin, M. J., Davie, J. R., Peterson, C. L. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science*, 2006, **311**:844-847, doi:10.1126/science.1124000

8. Choudhary, C., Kumar, C., Gnad, F., Nielsen, M. L., Rehman, M., Walther, T. C., et al. Lysine acetylation targets protein complexes and co-regulates major cellular functions. *Science*, 2009, **325**:834-840, doi:10.1126/science.1175371

9. Yang, X. J., Gregoire, S. Metabolism, cytoskeleton and cellular signalling in the grip of protein Nepsilon - and O-acetylation. *EMBO Rep*, 2007, **8**:556-562, doi:10.1038/sj.embor.7400977

10. Joanna, F., van Grunsven, L. A., Mathieu, V., Sarah, S., Sarah, D., Karin, V., et al. Histone deacetylase inhibition and the regulation of cell growth with particular reference to liver pathobiology. *J Cell Mol Med*, 2009, **13**:2990-3005, doi:10.1111/j.1582-4934.2009.00831.x

11. Chevallier, N., Corcoran, C. M., Lennon, C., Hyjek, E., Chadburn, A., Bardwell, V. J., et al. ETO protein of t(8;21) AML is a corepressor for Bcl-6 B-cell lymphoma oncoprotein. *Blood*, 2004, **103**:1454-1463, doi:10.1182/blood-2003-06-2081

12. Huang, B. H., Laban, M., Leung, C. H., Lee, L., Lee, C. K., Salto-Tellez, M., et al. Inhibition of histone deacetylase 2 increases apoptosis and p21Cip1/WAF1 expression, independent of histone deacetylase 1. *Cell Death Differ*, 2005, **12**:395-404, doi:10.1038/sj.cdd.4401567

13. Song, J., Noh, J. H., Lee, J. H., Eun, J. W., Ahn, Y. M., Kim, S. Y., et al. Increased expression of histone deacetylase 2 is found in human gastric cancer. *APMIS*, 2005, **113**:264-268, doi:10.1111/j.1600-0463.2005.apm_04.x

14. Lagger, G., Doetzlhofer, A., Schuettengruber, B., Haidweger, E., Simboeck, E., Tischler, J., et al. The tumor suppressor p53 and histone deacetylase 1 are antagonistic regulators of the cyclin-dependent kinase inhibitor p21/WAF1/CIP1 gene. *Mol Cell Biol*, 2003, **23**:2669-2679,

15. Zupkovitz, G., Grausenburger, R., Brunmeir, R., Senese, S., Tischler, J., Jurkin, J., et al. The cyclin-dependent kinase inhibitor p21 is a crucial target for histone deacetylase 1 as a regulator of cellular proliferation. *Mol Cell Biol*, 2010, **30**:1171-1181, doi:10.1128/MCB.01500-09

16. Wilson, A. J., Byun, D. S., Nasser, S., Murray, L. B., Ayyanar, K., Arango, D., et al. HDAC4 promotes growth of colon cancer cells via repression of p21. *Mol Biol Cell*, 2008, **19**:4062-4075, doi:10.1091/mbc.E08-02-0139

17. Mottet, D., Pirotte, S., Lamour, V., Hagedorn, M., Javerzat, S., Bikfalvi, A., et al. HDAC4 represses p21(WAF1/Cip1) expression in human cancer cells through a Sp1-dependent, p53-independent mechanism. *Oncogene*, 2009, **28**:243-256, doi:10.1038/onc.2008.371

18. Patra, S. K., Patra, A., Dahiya, R. Histone deacetylase and DNA methyltransferase in human prostate cancer. *Biochem Biophys Res Commun*, 2001, **287**:705-713, doi:10.1006/bbrc.2001.5639

19. Patra, S. K., Deb, M., Patra, A. Molecular marks for epigenetic identification of developmental and cancer stem cells. *Clin Epigenetics*, 2011, **2**:27-53, doi:10.1007/s13148-010-0016-0

20. Halkidou, K., Gaughan, L., Cook, S., Leung, H. Y., Neal, D. E., Robson, C. N. Upregulation and nuclear recruitment of HDAC1 in hormone refractory prostate cancer. *Prostate*, 2004, **59**:177-189, doi:10.1002/pros.20022

21. Weichert, W., Roske, A., Gekeler, V., Beckers, T., Stephan, C., Jung, K., et al. Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. *Br J Cancer*, 2008, **98**:604-610, doi:10.1038/sj.bjc.6604199

22. Velcich, A., Yang, W., Heyer, J., Fragale, A., Nicholas, C., Viani, S., et al. Colorectal cancer in mice genetically deficient in the mucin Muc2. *Science*, 2002, **295**:1726-1729, doi:10.1126/science.1069094

23. Yamada, N., Hamada, T., Goto, M., Tsutsumida, H., Higashi, M., Nomoto, M., et al. MUC2 expression is regulated by histone H3 modification and DNA methylation in pancreatic cancer. *Int J Cancer*, 2006, **119**:1850-1857, doi:10.1002/ijc.22047

24. Hatayama, H., Iwashita, J., Kuwajima, A., Abe, T. The short chain fatty acid, butyrate, stimulates MUC2 mucin production in the human colon cancer cell line, LS174T. *Biochem Biophys Res Commun*, 2007, **356**:599-603, doi:10.1016/j.bbrc.2007.03.025

25. Burger-van Paassen, N., Vincent, A., Puiman, P. J., van der Sluis, M., Bouma, J., Boehm, G., et al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem J*, 2009, **420**:211-219, doi:10.1042/BJ20082222

26. Schuler, S., Fritsche, P., Diersch, S., Arlt, A., Schmid, R. M., Saur, D., et al. HDAC2 attenuates TRAIL-induced apoptosis of pancreatic cancer cells. *Mol Cancer*, 2010, **9**:80, doi:10.1186/1476-4598-9-80

27. Fritsche, P., Seidler, B., Schuler, S., Schnieke, A., Gottlicher, M., Schmid, R. M., et al. HDAC2 mediates therapeutic resistance of pancreatic cancer cells via the BH3-only protein NOXA. *Gut*, 2009, **58**:1399-1409, doi:10.1136/gut.2009.180711

28. Senese, S., Zaragoza, K., Minardi, S., Muradore, I., Ronzoni, S., Passafaro, A., et al. Role for histone deacetylase 1 in human tumor cell proliferation. *Mol Cell Biol*, 2007, **27**:4784-4795, doi:10.1128/MCB.00494-07

29. Kaminskyy, V. O., Surova, O. V., Vaculova, A., Zhivotovsky, B. Combined inhibition of DNA methyltransferase and histone deacetylase restores caspase-8 expression and sensitizes SCLC cells to TRAIL. *Carcinogenesis*, 2011, **32**:1450-1458, doi:10.1093/carcin/bgr135

30. Aguilera, D. G., Das, C. M., Sinnappah-Kang, N. D., Joyce, C.,

Taylor, P. H., Wen, S., et al. Reactivation of death receptor 4 (DR4) expression sensitizes medulloblastoma cell lines to TRAIL. *J Neurooncol*, 2009, **93**:303-318, doi:10.1007/s11060-008-9788-x
31. Jung, K. H., Noh, J. H., Kim, J. K., Eun, J. W., Bae, H. J., Xie, H. J., et al. HDAC2 overexpression confers oncogenic potential to human lung cancer cells by deregulating expression of apoptosis and cell cycle proteins. *J Cell Biochem*, 2012, **113**:2167-2177, doi:10.1002/jcb.24090
32. Butler, L. M., Liapis, V., Bouralexis, S., Welldon, K., Hay, S., Thai le, M., et al. The histone deacetylase inhibitor, suberoylanilide hydroxamic acid, overcomes resistance of human breast cancer cells to Apo2L/TRAIL. *Int J Cancer*, 2006, **119**:944-954, doi:10.1002/ijc.21939
33. Shankar, S., Davis, R., Singh, K. P., Kurzrock, R., Ross, D. D., Srivastava, R. K. Suberoylanilide hydroxamic acid (Zolinza/vorinostat) sensitizes TRAIL-resistant breast cancer cells orthotopically implanted in BALB/c nude mice. *Mol Cancer Ther*, 2009, **8**:1596-1605, doi:10.1158/1535-7163.MCT-08-1004
34. Peixoto, P., Castronovo, V., Matheus, N., Polese, C., Peulen, O., Gonzalez, A., et al. HDAC5 is required for maintenance of pericentric heterochromatin, and controls cell-cycle progression and survival of human cancer cells. *Cell Death Differ*, 2012, **19**:1239-1252, doi:10.1038/cdd.2012.3
35. Yasui, W., Oue, N., Ono, S., Mitani, Y., Ito, R., Nakayama, H. Histone acetylation and gastrointestinal carcinogenesis. *Ann N Y Acad Sci*, 2003, **983**:220-231,
36. Geng, H., Harvey, C. T., Pittsenbarger, J., Liu, Q., Beer, T. M., Xue, C., et al. HDAC4 protein regulates HIF1alpha protein lysine acetylation and cancer cell response to hypoxia. *J Biol Chem*, 2011, **286**:38095-38102, doi:10.1074/jbc.M111.257055
37. Obach, M., Navarro-Sabate, A., Caro, J., Kong, X., Duran, J., Gomez, M., et al. 6-Phosphofructo-2-kinase (pfkfb3) gene promoter contains hypoxia-inducible factor-1 binding sites necessary for transactivation in response to hypoxia. *J Biol Chem*, 2004, **279**:53562-53570, doi:10.1074/jbc.M406096200
38. Kress, S., Stein, A., Maurer, P., Weber, B., Reichert, J., Buchmann, A., et al. Expression of hypoxia-inducible genes in tumor cells. *J Cancer Res Clin Oncol*, 1998, **124**:315-320,
39. Liu, Y., Li, Y. M., Tian, R. F., Liu, W. P., Fei, Z., Long, Q. F., et al. The expression and significance of HIF-1alpha and GLUT-3 in glioma. *Brain Res*, 2009, **1304**:149-154, doi:10.1016/j.brainres.2009.09.083
40. Eot-Houllier, G., Fulcrand, G., Magnaghi-Jaulin, L., Jaulin, C. Histone deacetylase inhibitors and genomic instability. *Cancer Lett*, 2009, **274**:169-176, doi:10.1016/j.canlet.2008.06.005
41. Kim, I. A., Kim, J. H., Shin, J. H., Kim, I. H., Kim, J. S., Wu, H. G., et al. A histone deacetylase inhibitor, trichostatin A, enhances radiosensitivity by abrogating G2/M arrest in human carcinoma cells. *Cancer Res Treat*, 2005, **37**:122-128, doi:10.4143/crt.2005.37.2.122
42. Miller, T. A., Witter, D. J., Belvedere, S. Histone deacetylase inhibitors. *J Med Chem*, 2003, **46**:5097-5116, doi:10.1021/jm0303094
43. Schemies, J., Sippl, W., Jung, M. Histone deacetylase inhibitors that target tubulin. *Cancer Lett*, 2009, **280**:222-232, doi:10.1016/j.canlet.2009.01.040
44. North, B. J., Verdin, E. Sirtuins: Sir2-related NAD-dependent protein deacetylases. *Genome Biol*, 2004, **5**:224, doi:10.1186/gb-2004-5-5-224
45. Westphal, C. H., Dipp, M. A., Guarente, L. A therapeutic role for sirtuins in diseases of aging? *Trends Biochem Sci*, 2007, **32**:555-560, doi:10.1016/j.tibs.2007.09.008
46. Fatkins, D. G., Zheng, W. Substituting N(epsilon)-thioacetyllysine for N(epsilon)-acetyl-lysine in peptide substrates as a general approach to inhibiting human NAD(+)-dependent protein deacetylases. *Int J Mol Sci*, 2008, **9**:1-11,
47. Biel, M., Kretsovali, A., Karatzali, E., Papamatheakis, J., Giannis, A. Design, synthesis, and biological evaluation of a small-molecule inhibitor of the histone acetyltransferase Gcn5. *Angew Chem Int Ed Engl*, 2004, **43**:3974-3976, doi:10.1002/anie.200453879
48. Solomon, J. M., Pasupuleti, R., Xu, L., McDonagh, T., Curtis, R., DiStefano, P. S., et al. Inhibition of SIRT1 catalytic activity increases p53 acetylation but does not alter cell survival following DNA damage. *Mol Cell Biol*, 2006, **26**:28-38, doi:10.1128/MCB.26.1.28-38.2006
49. Gui, C. Y., Ngo, L., Xu, W. S., Richon, V. M., Marks, P. A. Histone deacetylase (HDAC) inhibitor activation of p21WAF1 involves changes in promoter-associated proteins, including HDAC1. *Proc Natl Acad Sci U S A*, 2004, **101**:1241-1246, doi:10.1073/pnas.0307708100
50. Medda, F., Russell, R. J., Higgins, M., McCarthy, A. R., Campbell, J., Slawin, A. M., et al. Novel cambinol analogs as sirtuin inhibitors: synthesis, biological evaluation, and rationalization of activity. *J Med Chem*, 2009, **52**:2673-2682, doi:10.1021/jm8014298
51. Sandor, V., Senderowicz, A., Mertins, S., Sackett, D., Sausville, E., Blagosklonny, M. V., et al. P21-dependent g(1)arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228. *Br J Cancer*, 2000, **83**:817-825, doi:10.1054/bjoc.2000.1327
52. Wharton, W., Savell, J., Cress, W. D., Seto, E., Pledger, W. J. Inhibition of mitogenesis in Balb/c-3T3 cells by Trichostatin A. Multiple alterations in the induction and activation of cyclin-cyclin-dependent kinase complexes. *J Biol Chem*, 2000, **275**:33981-33987, doi:10.1074/jbc.M005600200
53. Bolden, J. E., Peart, M. J., Johnstone, R. W. Anticancer activities of histone deacetylase inhibitors. *Nat Rev Drug Discov*, 2006, **5**:769-784, doi:10.1038/nrd2133
54. Espino, P. S., Drobic, B., Dunn, K. L., Davie, J. R. Histone modifications as a platform for cancer therapy. *J Cell Biochem*, 2005, **94**:1088-1102, doi:10.1002/jcb.20387
55. Tu, C. Y., Chen, C. H., Hsia, T. C., Hsu, M. H., Wei, Y. L., Yu, M. C., et al. Trichostatin A suppresses EGFR expression through induction of microRNA-7 in an HDAC-independent manner in lapatinib-treated cells. *Biomed Res Int*, 2014, **2014**:168949, doi:10.1155/2014/168949
56. Kelly, W. K., Richon, V. M., O'Connor, O., Curley, T., MacGregor-Curtelli, B., Tong, W., et al. Phase I clinical trial of histone deacetylase inhibitor: suberoylanilide hydroxamic acid administered intravenously. *Clin Cancer Res*, 2003, **9**:3578-3588,
57. Bhalla, K. N. Epigenetic and chromatin modifiers as targeted therapy of hematologic malignancies. *J Clin Oncol*, 2005, **23**:3971-3993, doi:10.1200/JCO.2005.16.600
58. Dokmanovic, M., Marks, P. A. Prospects: histone deacetylase inhibitors. *J Cell Biochem*, 2005, **96**:293-304, doi:10.1002/jcb.20532
59. Marchion, D. C., Bicaku, E., Daud, A. I., Richon, V., Sullivan, D. M., Munster, P. N. Sequence-specific potentiation of topoisomerase II inhibitors by the histone deacetylase inhibitor suberoylanilide hydroxamic acid. *J Cell Biochem*, 2004, **92**:223-237, doi:10.1002/jcb.20045
60. Gore, S. D., Baylin, S., Sugar, E., Carraway, H., Miller, C. B., Carducci, M., et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res*, 2006, **66**:6361-6369, doi:10.1158/0008-5472.CAN-06-0080
61. Maslak, P., Chanel, S., Camacho, L. H., Soignet, S., Pandolfi, P. P., Guernah, I., et al. Pilot study of combination transcriptional modulation therapy with sodium phenylbutyrate and 5-azacytidine in patients with acute myeloid leukemia or myelodysplastic syndrome. *Leukemia*, 2006, **20**:212-217, doi:10.1038/sj.leu.2404050
62. Garcia-Manero, G., Kantarjian, H. M., Sanchez-Gonzalez, B.,

Yang, H., Rosner, G., Verstovsek, S., et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. *Blood*, 2006, **108**:3271-3279, doi:10.1182/blood-2006-03-009142

63. Nemunaitis, J. J., Orr, D., Eager, R., Cunningham, C. C., Williams, A., Mennel, R., et al. Phase I study of oral CI-994 in combination with gemcitabine in treatment of patients with advanced cancer. *Cancer J*, 2003, **9**:58-66,

64. Undevia, S. D., Kindler, H. L., Janisch, L., Olson, S. C., Schilsky, R. L., Vogelzang, N. J., et al. A phase I study of the oral combination of CI-994, a putative histone deacetylase inhibitor, and capecitabine. *Ann Oncol*, 2004, **15**:1705-1711, doi:10.1093/annonc/mdh438

65. Yu, C., Rahmani, M., Conrad, D., Subler, M., Dent, P., Grant, S. The proteasome inhibitor bortezomib interacts synergistically with histone deacetylase inhibitors to induce apoptosis in Bcr/Abl+ cells sensitive and resistant to STI571. *Blood*, 2003, **102**:3765-3774, doi:10.1182/blood-2003-03-0737

66. Fiskus, W., Pranpat, M., Bali, P., Balasis, M., Kumaraswamy, S., Boyapalle, S., et al. Combined effects of novel tyrosine kinase inhibitor AMN107 and histone deacetylase inhibitor LBH589 against Bcr-Abl-expressing human leukemia cells. *Blood*, 2006, **108**:645-652, doi:10.1182/blood-2005-11-4639

67. Chen, Z. X., Mann, J. R., Hsieh, C. L., Riggs, A. D., Chedin, F. Physical and functional interactions between the human DNMT3L protein and members of the de novo methyltransferase family. *J Cell Biochem*, 2005, **95**:902-917, doi:10.1002/jcb.20447

68. Allfrey, V. G., Faulkner, R., Mirsky, A. E. Acetylation and Methylation of Histones and Their Possible Role in the Regulation of Rna Synthesis. *Proc Natl Acad Sci U S A*, 1964, **51**:786-794,

69. Kleff, S., Andrulis, E. D., Anderson, C. W., Sternglanz, R. Identification of a gene encoding a yeast histone H4 acetyltransferase. *J Biol Chem*, 1995, **270**:24674-24677,

70. Allis, C. D., Chicoine, L. G., Richman, R., Schulman, I. G. Deposition-related histone acetylation in micronuclei of conjugating Tetrahymena. *Proc Natl Acad Sci U S A*, 1985, **82**:8048-8052,

71. Ruiz-Carrillo, A., Wangh, L. J., Allfrey, V. G. Processing of newly synthesized histone molecules. *Science*, 1975, **190**:117-128,

72. Parthun, M. R., Widom, J., Gottschling, D. E. The major cytoplasmic histone acetyltransferase in yeast: links to chromatin replication and histone metabolism. *Cell*, 1996, **87**:85-94,

73. Yuan, H., Marmorstein, R. Histone acetyltransferases: Rising ancient counterparts to protein kinases. *Biopolymers*, 2013, **99**:98-111, doi:10.1002/bip.22128

74. Cullis, P. M., Wolfenden, R., Cousens, L. S., Alberts, B. M. Inhibition of histone acetylation by N-[2-(S-coenzyme A)acetyl] spermidine amide, a multisubstrate analog. *J Biol Chem*, 1982, **257**:12165-12169,

75. Balasubramanyam, K., Altaf, M., Varier, R. A., Swaminathan, V., Ravindran, A., Sadhale, P. P., et al. Polyisoprenylated benzophenone, garcinol, a natural histone acetyltransferase inhibitor, represses chromatin transcription and alters global gene expression. *J Biol Chem*, 2004, **279**:33716-33726, doi:10.1074/jbc.M402839200

76. Balasubramanyam, K., Varier, R. A., Altaf, M., Swaminathan, V., Siddappa, N. B., Ranga, U., et al. Curcumin, a novel p300/CREB-binding protein-specific inhibitor of acetyltransferase, represses the acetylation of histone/nonhistone proteins and histone acetyltransferase-dependent chromatin transcription. *J Biol Chem*, 2004, **279**:51163-51171, doi:10.1074/jbc.M409024200

77. Stimson, L., Rowlands, M. G., Newbatt, Y. M., Smith, N. F., Raynaud, F. I., Rogers, P., et al. Isothiazolones as inhibitors of PCAF and p300 histone acetyltransferase activity. *Mol Cancer Ther*, 2005, **4**:1521-1532, doi:10.1158/1535-7163.MCT-05-0135

78. Nagy, Z., Tora, L. Distinct GCN5/PCAF-containing complexes function as co-activators and are involved in transcription factor and global histone acetylation. *Oncogene*, 2007, **26**:5341-5357, doi:10.1038/sj.onc.1210604

79. Sun, Y., Jiang, X., Chen, S., Fernandes, N., Price, B. D. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. *Proc Natl Acad Sci U S A*, 2005, **102**:13182-13187, doi:10.1073/pnas.0504211102

80. Black, J. C., Van Rechem, C., Whetstine, J. R. Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol Cell*, 2012, **48**:491-507, doi:10.1016/j.molcel.2012.11.006S1097-2765(12)00937-9 [pii]

81. Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature*, 2000, **406**:593-599, doi:10.1038/35020506

82. Jenuwein, T. The epigenetic magic of histone lysine methylation. *FEBS J*, 2006, **273**:3121-3135, doi:10.1111/j.1742-4658.2006.05343.x

83. Dillon, S. C., Zhang, X., Trievel, R. C., Cheng, X. The SET-domain protein superfamily: protein lysine methyltransferases. *Genome Biol*, 2005, **6**:227, doi:10.1186/gb-2005-6-8-227

84. Okada, Y., Feng, Q., Lin, Y., Jiang, Q., Li, Y., Coffield, V. M., et al. hDOT1L links histone methylation to leukemogenesis. *Cell*, 2005, **121**:167-178, doi:10.1016/j.cell.2005.02.020

85. van Leeuwen, F., Gafken, P. R., Gottschling, D. E. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell*, 2002, **109**:745-756,

86. Nguyen, A. T., Zhang, Y. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev*, 2011, **25**:1345-1358, doi:10.1101/gad.2057811

87. Shi, Y., Sawada, J., Sui, G., Affar el, B., Whetstine, J. R., Lan, F., et al. Coordinated histone modifications mediated by a CtBP co-repressor complex. *Nature*, 2003, **422**:735-738, doi:10.1038/nature01550

88. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell*, 2004, **119**:941-953, doi:10.1016/j.cell.2004.12.012

89. Yamane, K., Toumazou, C., Tsukada, Y., Erdjument-Bromage, H., Tempst, P., Wong, J., et al. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. *Cell*, 2006, **125**:483-495, doi:10.1016/j.cell.2006.03.027

90. Tsukada, Y., Fang, J., Erdjument-Bromage, H., Warren, M. E., Borchers, C. H., Tempst, P., et al. Histone demethylation by a family of JmjC domain-containing proteins. *Nature*, 2006, **439**:811-816, doi:10.1038/nature04433

91. Shi, Y., Whetstine, J. R. Dynamic regulation of histone lysine methylation by demethylases. *Mol Cell*, 2007, **25**:1-14, doi:10.1016/j.molcel.2006.12.010

92. Klose, R. J., Yamane, K., Bae, Y., Zhang, D., Erdjument-Bromage, H., Tempst, P., et al. The transcriptional repressor JHDM3A demethylates trimethyl histone H3 lysine 9 and lysine 36. *Nature*, 2006, **442**:312-316, doi:10.1038/nature04853

93. Fodor, B. D., Kubicek, S., Yonezawa, M., O'Sullivan, R. J., Sengupta, R., Perez-Burgos, L., et al. Jmjd2b antagonizes H3K9 trimethylation at pericentric heterochromatin in mammalian cells. *Genes Dev*, 2006, **20**:1557-1562, doi:10.1101/gad.388206

94. Cloos, P. A., Christensen, J., Agger, K., Maiolica, A., Rappsilber, J., Antal, T., et al. The putative oncogene GASC1 demethylates tri- and dimethylated lysine 9 on histone H3. *Nature*, 2006, **442**:307-311, doi:10.1038/nature04837

95. Whetstine, J. R., Nottke, A., Lan, F., Huarte, M., Smolikov, S., Chen, Z., et al. Reversal of histone lysine trimethylation by the JMJD2 family of histone demethylases. *Cell*, 2006, **125**:467-481,

doi:10.1016/j.cell.2006.03.028
96. Trojer, P., Zhang, J., Yonezawa, M., Schmidt, A., Zheng, H., Jenuwein, T., et al. Dynamic Histone H1 Isotype 4 Methylation and Demethylation by Histone Lysine Methyltransferase G9a/KMT1C and the Jumonji Domain-containing JMJD2/KDM4 Proteins. *J Biol Chem*, 2009, **284**:8395-8405, doi:10.1074/jbc.M807818200
97. Kouzarides, T. Chromatin modifications and their function. *Cell*, 2007, **128**:693-705, doi:10.1016/j.cell.2007.02.005
98. Trojer, P., Reinberg, D. Facultative heterochromatin: is there a distinctive molecular signature? *Mol Cell*, 2007, **28**:1-13, doi:10.1016/j.molcel.2007.09.011
99. Vermeulen, M., Mulder, K. W., Denissov, S., Pijnappel, W. W., van Schaik, F. M., Varier, R. A., et al. Selective anchoring of TFIID to nucleosomes by trimethylation of histone H3 lysine 4. *Cell*, 2007, **131**:58-69, doi:10.1016/j.cell.2007.08.016
100. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., Jenuwein, T. Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature*, 2001, **410**:116-120, doi:10.1038/35065132
101. Sarris, M., Nikolaou, K., Talianidis, I. Context-specific regulation of cancer epigenomes by histone and transcription factor methylation. *Oncogene*, 2014, **33**:1207-1217, doi:10.1038/onc.2013.87onc201387 [pii]
102. Huen, M. S., Sy, S. M., van Deursen, J. M., Chen, J. Direct interaction between SET8 and proliferating cell nuclear antigen couples H4-K20 methylation with DNA replication. *J Biol Chem*, 2008, **283**:11073-11077, doi:10.1074/jbc.C700242200
103. Jorgensen, S., Elvers, I., Trelle, M. B., Menzel, T., Eskildsen, M., Jensen, O. N., et al. The histone methyltransferase SET8 is required for S-phase progression. *J Cell Biol*, 2007, **179**:1337-1345, doi:10.1083/jcb.200706150
104. Rice, J. C., Nishioka, K., Sarma, K., Steward, R., Reinberg, D., Allis, C. D. Mitotic-specific methylation of histone H4 Lys 20 follows increased PR-Set7 expression and its localization to mitotic chromosomes. *Genes Dev*, 2002, **16**:2225-2230, doi:10.1101/gad.1014902
105. Jorgensen, S., Eskildsen, M., Fugger, K., Hansen, L., Larsen, M. S., Kousholt, A. N., et al. SET8 is degraded via PCNA-coupled CRL4(CDT2) ubiquitylation in S phase and after UV irradiation. *J Cell Biol*, 2011, **192**:43-54, doi:10.1083/jcb.201009076
106. Tardat, M., Brustel, J., Kirsh, O., Lefevbre, C., Callanan, M., Sardet, C., et al. The histone H4 Lys 20 methyltransferase PR-Set7 regulates replication origins in mammalian cells. *Nat Cell Biol*, 2010, **12**:1086-1093, doi:10.1038/ncb2113
107. Beck, D. B., Burton, A., Oda, H., Ziegler-Birling, C., Torres-Padilla, M. E., Reinberg, D. The role of PR-Set7 in replication licensing depends on Suv4-20h. *Genes Dev*, 2012, **26**:2580-2589, doi:10.1101/gad.195636.112
108. Botuyan, M. V., Lee, J., Ward, I. M., Kim, J. E., Thompson, J. R., Chen, J., et al. Structural basis for the methylation state-specific recognition of histone H4-K20 by 53BP1 and Crb2 in DNA repair. *Cell*, 2006, **127**:1361-1373, doi:10.1016/j.cell.2006.10.043
109. Chou, R. H., Wang, Y. N., Hsieh, Y. H., Li, L. Y., Xia, W., Chang, W. C., et al. EGFR modulates DNA synthesis and repair through Tyr phosphorylation of histone H4. *Dev Cell*, 2014, **30**:224-237, doi:10.1016/j.devcel.2014.06.008
110. Schotta, G., Lachner, M., Sarma, K., Ebert, A., Sengupta, R., Reuter, G., et al. A silencing pathway to induce H3-K9 and H4-K20 trimethylation at constitutive heterochromatin. *Genes Dev*, 2004, **18**:1251-1262, doi:10.1101/gad.300704
111. Yang, F., Sun, L., Li, Q., Han, X., Lei, L., Zhang, H., et al. SET8 promotes epithelial-mesenchymal transition and confers TWIST dual transcriptional activities. *EMBO J*, 2012, **31**:110-123, doi:10.1038/emboj.2011.364
112. Feng, Q., Wang, H., Ng, H. H., Erdjument-Bromage, H., Tempst, P., Struhl, K., et al. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. *Curr Biol*, 2002, **12**:1052-1058,
113. Min, J., Feng, Q., Li, Z., Zhang, Y., Xu, R. M. Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell*, 2003, **112**:711-723,
114. Vidgren, J., Svensson, L. A., Liljas, A. Crystal structure of catechol O-methyltransferase. *Nature*, 1994, **368**:354-358, doi:10.1038/368354a0
115. Barry, E. R., Corry, G. N., Rasmussen, T. P. Targeting DOT1L action and interactions in leukemia: the role of DOT1L in transformation and development. *Expert Opin Ther Targets*, 2010, **14**:405-418, doi:10.1517/14728221003623241
116. Bernt, K. M., Armstrong, S. A. A role for DOT1L in MLL-rearranged leukemias. *Epigenomics*, 2011, **3**:667-670, doi:10.2217/epi.11.98
117. Deshpande, A. J., Chen, L., Fazio, M., Sinha, A. U., Bernt, K. M., Banka, D., et al. Leukemic transformation by the MLL-AF6 fusion oncogene requires the H3K79 methyltransferase Dot1l. *Blood*, 2013, **121**:2533-2541, doi:10.1182/blood-2012-11-465120
118. Sauvageau, M., Sauvageau, G. Polycomb group genes: keeping stem cell activity in balance. *PLoS Biol*, 2008, **6**:e113, doi:10.1371/journal.pbio.0060113
119. Choudhury, S. R., Balasubramanian, S., Chew, Y. C., Han, B., Marquez, V. E., Eckert, R. L. (-)-Epigallocatechin-3-gallate and DZNep reduce polycomb protein level via a proteasome-dependent mechanism in skin cancer cells. *Carcinogenesis*, 2011, **32**:1525-1532, doi:10.1093/carcin/bgr171
120. Simon, J. A., Lange, C. A. Roles of the EZH2 histone methyltransferase in cancer epigenetics. *Mutat Res*, 2008, **647**:21-29, doi:10.1016/j.mrfmmm.2008.07.010
121. Yoo, K. H., Hennighausen, L. EZH2 methyltransferase and H3K27 methylation in breast cancer. *Int J Biol Sci*, 2012, **8**:59-65,
122. Suva, M. L., Riggi, N., Janiszewska, M., Radovanovic, I., Provero, P., Stehle, J. C., et al. EZH2 is essential for glioblastoma cancer stem cell maintenance. *Cancer Res*, 2009, **69**:9211-9218, doi:10.1158/0008-5472.CAN-09-1622
123. Crea, F., Hurt, E. M., Farrar, W. L. Clinical significance of Polycomb gene expression in brain tumors. *Mol Cancer*, 2010, **9**:265, doi:10.1186/1476-4598-9-265
124. Orzan, F., Pellegatta, S., Poliani, P. L., Pisati, F., Caldera, V., Menghi, F., et al. Enhancer of Zeste 2 (EZH2) is up-regulated in malignant gliomas and in glioma stem-like cells. *Neuropathol Appl Neurobiol*, 2011, **37**:381-394, doi:10.1111/j.1365-2990.2010.01132.x
125. Li, G., Warden, C., Zou, Z., Neman, J., Krueger, J. S., Jain, A., et al. Altered expression of polycomb group genes in glioblastoma multiforme. *PLoS One*, 2013, **8**:e80970, doi:10.1371/journal.pone.0080970
126. Chou, R. H., Yu, Y. L., Hung, M. C. The roles of EZH2 in cell lineage commitment. *Am J Transl Res*, 2011, **3**:243-250,
127. Yu, Y. L., Chou, R. H., Chen, L. T., Shyu, W. C., Hsieh, S. C., Wu, C. S., et al. EZH2 regulates neuronal differentiation of mesenchymal stem cells through PIP5K1C-dependent calcium signaling. *J Biol Chem*, 2011, **286**:9657-9667, doi:10.1074/jbc.M110.185124
128. Yu, Y. L., Chou, R. H., Shyu, W. C., Hsieh, S. C., Wu, C. S., Chiang, S. Y., et al. Smurf2-mediated degradation of EZH2 enhances neuron differentiation and improves functional recovery after ischaemic stroke. *EMBO Mol Med*, 2013, **5**:531-547, doi:10.1002/emmm.201201783
129. Chou, R. H., Chiu, L., Yu, Y. L., Shyu, W. C. The potential roles of EZH2 in regenerative medicine. *Cell Transplant*, 2015, **24**:313-317, doi:10.3727/096368915X686823
130. He, L. R., Liu, M. Z., Li, B. K., Jia, W. H., Zhang, Y., Liao, Y. J., et al. High expression of EZH2 is associated with tumor aggres-

siveness and poor prognosis in patients with esophageal squamous cell carcinoma treated with definitive chemoradiotherapy. *Int J Cancer*, 2010, **127**:138-147, doi:10.1002/ijc.25031

131. Hu, S., Yu, L., Li, Z., Shen, Y., Wang, J., Cai, J., et al. Overexpression of EZH2 contributes to acquired cisplatin resistance in ovarian cancer cells in vitro and in vivo. *Cancer Biol Ther*, 2010, **10**:788-795,

132. Rizzo, S., Hersey, J. M., Mellor, P., Dai, W., Santos-Silva, A., Liber, D., et al. Ovarian cancer stem cell-like side populations are enriched following chemotherapy and overexpress EZH2. *Mol Cancer Ther*, 2011, **10**:325-335, doi:10.1158/1535-7163.MCT-10-0788

133. Chang, C. J., Yang, J. Y., Xia, W., Chen, C. T., Xie, X., Chao, C. H., et al. EZH2 promotes expansion of breast tumor initiating cells through activation of RAF1-beta-catenin signaling. *Cancer Cell*, 2011, **19**:86-100, doi:S1535-6108(10)00437-X [pii] 10.1016/j.ccr.2010.10.035

134. Kong, D., Heath, E., Chen, W., Cher, M. L., Powell, I., Heilbrun, L., et al. Loss of let-7 up-regulates EZH2 in prostate cancer consistent with the acquisition of cancer stem cell signatures that are attenuated by BR-DIM. *PLoS One*, 2012, **7**:e33729, doi:10.1371/journal.pone.0033729

135. Reijm, E. A., Jansen, M. P., Ruigrok-Ritstier, K., van Staveren, I. L., Look, M. P., van Gelder, M. E., et al. Decreased expression of EZH2 is associated with upregulation of ER and favorable outcome to tamoxifen in advanced breast cancer. *Breast Cancer Res Treat*, 2011, **125**:387-394, doi:10.1007/s10549-010-0836-9

136. Jansen, M. P., Reijm, E. A., Sieuwerts, A. M., Ruigrok-Ritstier, K., Look, M. P., Rodriguez-Gonzalez, F. G., et al. High miR-26a and low CDC2 levels associate with decreased EZH2 expression and with favorable outcome on tamoxifen in metastatic breast cancer. *Breast Cancer Res Treat*, 2012, **133**:937-947, doi:10.1007/s10549-011-1877-4

137. Zhang, Y., Liu, G., Lin, C., Liao, G., Tang, B. Silencing the EZH2 gene by RNA interference reverses the drug resistance of human hepatic multidrug-resistant cancer cells to 5-Fu. *Life Sci*, 2013, **92**:896-902, doi:10.1016/j.lfs.2013.03.010

138. Tang, B., Zhang, Y., Liang, R., Gao, Z., Sun, D., Wang, L. RNAi-mediated EZH2 depletion decreases MDR1 expression and sensitizes multidrug-resistant hepatocellular carcinoma cells to chemotherapy. *Oncol Rep*, 2013, **29**:1037-1042, doi:10.3892/or.2013.2222

139. Daigle, S. R., Olhava, E. J., Therkelsen, C. A., Majer, C. R., Sneeringer, C. J., Song, J., et al. Selective killing of mixed lineage leukemia cells by a potent small-molecule DOT1L inhibitor. *Cancer Cell*, 2011, **20**:53-65, doi:10.1016/j.ccr.2011.06.009

140. Chen, L., Deshpande, A. J., Banka, D., Bernt, K. M., Dias, S., Buske, C., et al. Abrogation of MLL-AF10 and CALM-AF10-mediated transformation through genetic inactivation or pharmacological inhibition of the H3K79 methyltransferase Dot1l. *Leukemia*, 2013, **27**:813-822, doi:10.1038/leu.2012.327

141. Gibbons, G. S., Owens, S. R., Fearon, E. R., Nikolovska-Coleska, Z. Regulation of Wnt signaling target gene expression by the histone methyltransferase DOT1L. *ACS Chem Biol*, 2015, **10**:109-114, doi:10.1021/cb500668u

142. Daigle, S. R., Olhava, E. J., Therkelsen, C. A., Basavapathruni, A., Jin, L., Boriack-Sjodin, P. A., et al. Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. *Blood*, 2013, **122**:1017-1025, doi:10.1182/blood-2013-04-497644

143. Basavapathruni, A., Olhava, E. J., Daigle, S. R., Therkelsen, C. A., Jin, L., Boriack-Sjodin, P. A., et al. Nonclinical pharmacokinetics and metabolism of EPZ-5676, a novel DOT1L histone methyltransferase inhibitor. *Biopharm Drug Dispos*, 2014, **35**:237-252, doi:10.1002/bdd.1889

144. Kuhn, M. W., Hadler, M. J., Daigle, S. R., Koche, R. P., Krivtsov, A. V., Olhava, E. J., et al. MLL partial tandem duplication leukemia cells are sensitive to small molecule DOT1L inhibition. *Haematologica*, 2015, **100**:e190-193, doi:10.3324/haematol.2014.115337

145. Klaus, C. R., Iwanowicz, D., Johnston, D., Campbell, C. A., Smith, J. J., Moyer, M. P., et al. DOT1L inhibitor EPZ-5676 displays synergistic antiproliferative activity in combination with standard of care drugs and hypomethylating agents in MLL-rearranged leukemia cells. *J Pharmacol Exp Ther*, 2014, **350**:646-656, doi:10.1124/jpet.114.214577

146. McCabe, M. T., Ott, H. M., Ganji, G., Korenchuk, S., Thompson, C., Van Aller, G. S., et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. *Nature*, 2012, **492**:108-112, doi:10.1038/nature11606

147. Qi, W., Chan, H., Teng, L., Li, L., Chuai, S., Zhang, R., et al. Selective inhibition of Ezh2 by a small molecule inhibitor blocks tumor cells proliferation. *Proc Natl Acad Sci U S A*, 2012, **109**:21360-21365, doi:10.1073/pnas.1210371110

148. Konze, K. D., Ma, A., Li, F., Barsyte-Lovejoy, D., Parton, T., Macnevin, C. J., et al. An orally bioavailable chemical probe of the Lysine Methyltransferases EZH2 and EZH1. *ACS Chem Biol*, 2013, **8**:1324-1334, doi:10.1021/cb400133j

149. Knutson, S. K., Kawano, S., Minoshima, Y., Warholic, N. M., Huang, K. C., Xiao, Y., et al. Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. *Mol Cancer Ther*, 2014, **13**:842-854, doi:10.1158/1535-7163.MCT-13-0773

150. Bradley, W. D., Arora, S., Busby, J., Balasubramanian, S., Gehling, V. S., Nasveschuk, C. G., et al. EZH2 inhibitor efficacy in non-Hodgkin's lymphoma does not require suppression of H3K27 monomethylation. *Chem Biol*, 2014, **21**:1463-1475, doi:10.1016/j.chembiol.2014.09.017

151. Velichutina, I., Shaknovich, R., Geng, H., Johnson, N. A., Gascoyne, R. D., Melnick, A. M., et al. EZH2-mediated epigenetic silencing in germinal center B cells contributes to proliferation and lymphomagenesis. *Blood*, 2010, **116**:5247-5255, doi:10.1182/blood-2010-04-280149

152. Beguelin, W., Popovic, R., Teater, M., Jiang, Y., Bunting, K. L., Rosen, M., et al. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. *Cancer Cell*, 2013, **23**:677-692, doi:10.1016/j.ccr.2013.04.011

153. Knutson, S. K., Warholic, N. M., Wigle, T. J., Klaus, C. R., Allain, C. J., Raimondi, A., et al. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. *Proc Natl Acad Sci U S A*, 2013, **110**:7922-7927, doi:10.1073/pnas.1303800110

154. Pappano, W. N., Guo, J., He, Y., Ferguson, D., Jagadeeswaran, S., Osterling, D. J., et al. The Histone Methyltransferase Inhibitor A-366 Uncovers a Role for G9a/GLP in the Epigenetics of Leukemia. *PLoS One*, 2015, **10**:e0131716, doi:10.1371/journal.pone.0131716

155. Kim, Y., Kim, Y. S., Kim, D. E., Lee, J. S., Song, J. H., Kim, H. G., et al. BIX-01294 induces autophagy-associated cell death via EHMT2/G9a dysfunction and intracellular reactive oxygen species production. *Autophagy*, 2013, **9**:2126-2139, doi:10.4161/auto.26308

156. Savickiene, J., Treigyte, G., Stirblyte, I., Valiuliene, G., Navakauskiene, R. Euchromatic histone methyltransferase 2 inhibitor, BIX-01294, sensitizes human promyelocytic leukemia HL-60 and NB4 cells to growth inhibition and differentiation. *Leuk Res*, 2014, **38**:822-829, doi:10.1016/j.leukres.2014.04.003

157. Cui, J., Sun, W., Hao, X., Wei, M., Su, X., Zhang, Y., et al. EHMT2 inhibitor BIX-01294 induces apoptosis through PMAIP1-USP9X-MCL1 axis in human bladder cancer cells. *Cancer Cell Int*, 2015, **15**:4, doi:10.1186/s12935-014-0149-x

158. Ferguson, A. D., Larsen, N. A., Howard, T., Pollard, H., Green,

I., Grande, C., et al. Structural basis of substrate methylation and inhibition of SMYD2. *Structure*, 2011, **19**:1262-1273, doi:10.1016/j.str.2011.06.011

159. Nguyen, H., Allali-Hassani, A., Antonysamy, S., Chang, S., Chen, L. H., Curtis, C., et al. LLY-507, a Cell-active, Potent, and Selective Inhibitor of Protein-lysine Methyltransferase SMYD2. *J Biol Chem*, 2015, **290**:13641-13653, doi:10.1074/jbc.M114.626861

160. Schmidt, D. M., McCafferty, D. G. trans-2-Phenylcyclopropylamine is a mechanism-based inactivator of the histone demethylase LSD1. *Biochemistry*, 2007, **46**:4408-4416, doi:10.1021/bi0618621

161. Ueda, R., Suzuki, T., Mino, K., Tsumoto, H., Nakagawa, H., Hasegawa, M., et al. Identification of cell-active lysine specific demethylase 1-selective inhibitors. *J Am Chem Soc*, 2009, **131**:17536-17537, doi:10.1021/ja907055q

162. Mimasu, S., Umezawa, N., Sato, S., Higuchi, T., Umehara, T., Yokoyama, S. Structurally designed trans-2-phenylcyclopropylamine derivatives potently inhibit histone demethylase LSD1/KDM1. *Biochemistry*, 2010, **49**:6494-6503, doi:10.1021/bi100299r

163. Benelkebir, H., Hodgkinson, C., Duriez, P. J., Hayden, A. L., Bulleid, R. A., Crabb, S. J., et al. Enantioselective synthesis of tranylcypromine analogues as lysine demethylase (LSD1) inhibitors. *Bioorg Med Chem*, 2011, **19**:3709-3716, doi:10.1016/j.bmc.2011.02.017

164. Harris, W. J., Huang, X., Lynch, J. T., Spencer, G. J., Hitchin, J. R., Li, Y., et al. The histone demethylase KDM1A sustains the oncogenic potential of MLL-AF9 leukemia stem cells. *Cancer Cell*, 2012, **21**:473-487, doi:10.1016/j.ccr.2012.03.014

165. Maes, T., Mascaro, C., Ortega, A., Lunardi, S., Ciceri, F., Somervaille, T. C., et al. KDM1 histone lysine demethylases as targets for treatments of oncological and neurodegenerative disease. *Epigenomics*, 2015, **7**:609-626, doi:10.2217/epi.15.9

166. Mohammad, H. P., Smitheman, K. N., Kamat, C. D., Soong, D., Federowicz, K. E., Van Aller, G. S., et al. A DNA Hypomethylation Signature Predicts Antitumor Activity of LSD1 Inhibitors in SCLC. *Cancer Cell*, 2015, **28**:57-69, doi:10.1016/j.ccell.2015.06.002

167. Sorna, V., Theisen, E. R., Stephens, B., Warner, S. L., Bearss, D. J., Vankayalapati, H., et al. High-throughput virtual screening identifies novel N'-(1-phenylethylidene)-benzohydrazides as potent, specific, and reversible LSD1 inhibitors. *J Med Chem*, 2013, **56**:9496-9508, doi:10.1021/jm400870h

168. Dhanak, D., Jackson, P. Development and classes of epigenetic drugs for cancer. *Biochem Biophys Res Commun*, 2014, **455**:58-69, doi:10.1016/j.bbrc.2014.07.006

169. Fiskus, W., Sharma, S., Shah, B., Portier, B. P., Devaraj, S. G., Liu, K., et al. Highly effective combination of LSD1 (KDM1A) antagonist and pan-histone deacetylase inhibitor against human AML cells. *Leukemia*, 2014, **28**:2155-2164, doi:10.1038/leu.2014.119

170. Hazeldine, S., Pachaiyappan, B., Steinbergs, N., Nowotarski, S., Hanson, A. S., Casero, R. A., Jr., et al. Low molecular weight amidoximes that act as potent inhibitors of lysine-specific demethylase 1. *J Med Chem*, 2012, **55**:7378-7391, doi:10.1021/jm3002845

171. Rose, N. R., Ng, S. S., Mecinovic, J., Lienard, B. M., Bello, S. H., Sun, Z., et al. Inhibitor scaffolds for 2-oxoglutarate-dependent histone lysine demethylases. *J Med Chem*, 2008, **51**:7053-7056, doi:10.1021/jm800936s

172. Kruidenier, L., Chung, C. W., Cheng, Z., Liddle, J., Che, K., Joberty, G., et al. A selective jumonji H3K27 demethylase inhibitor modulates the proinflammatory macrophage response. *Nature*, 2012, **488**:404-408, doi:10.1038/nature11262

173. Ntziachristos, P., Tsirigos, A., Welstead, G. G., Trimarchi, T., Bakogianni, S., Xu, L., et al. Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia. *Nature*, 2014, **514**:513-517, doi:10.1038/nature13605

174. Dyson, M. H., Thomson, S., Inagaki, M., Goto, H., Arthur, S. J., Nightingale, K., et al. MAP kinase-mediated phosphorylation of distinct pools of histone H3 at S10 or S28 via mitogen- and stress-activated kinase 1/2. *J Cell Sci*, 2005, **118**:2247-2259, doi:10.1242/jcs.02373

175. Choi, H. S., Choi, B. Y., Cho, Y. Y., Mizuno, H., Kang, B. S., Bode, A. M., et al. Phosphorylation of histone H3 at serine 10 is indispensable for neoplastic cell transformation. *Cancer Res*, 2005, **65**:5818-5827, doi:10.1158/0008-5472.CAN-05-0197

176. Dong, Z., Bode, A. M. The role of histone H3 phosphorylation (Ser10 and Ser28) in cell growth and cell transformation. *Mol Carcinog*, 2006, **45**:416-421, doi:10.1002/mc.20220

177. Espino, P. S., Pritchard, S., Heng, H. H., Davie, J. R. Genomic instability and histone H3 phosphorylation induction by the Ras-mitogen activated protein kinase pathway in pancreatic cancer cells. *Int J Cancer*, 2009, **124**:562-567, doi:10.1002/ijc.23959

178. Castellano-Pozo, M., Santos-Pereira, J. M., Rondon, A. G., Barroso, S., Andujar, E., Perez-Alegre, M., et al. R loops are linked to histone H3 S10 phosphorylation and chromatin condensation. *Mol Cell*, 2013, **52**:583-590, doi:10.1016/j.molcel.2013.10.006

179. Skourti-Stathaki, K., Proudfoot, N. J. Histone 3 s10 phosphorylation: «caught in the R loop!». *Mol Cell*, 2013, **52**:470-472, doi:10.1016/j.molcel.2013.11.006

180. Maile, T., Kwoczynski, S., Katzenberger, R. J., Wassarman, D. A., Sauer, F. TAF1 activates transcription by phosphorylation of serine 33 in histone H2B. *Science*, 2004, **304**:1010-1014, doi:10.1126/science.1095001

181. Metzger, E., Yin, N., Wissmann, M., Kunowska, N., Fischer, K., Friedrichs, N., et al. Phosphorylation of histone H3 at threonine 11 establishes a novel chromatin mark for transcriptional regulation. *Nat Cell Biol*, 2008, **10**:53-60, doi:10.1038/ncb1668

182. Xiao, A., Li, H., Shechter, D., Ahn, S. H., Fabrizio, L. A., Erdjument-Bromage, H., et al. WSTF regulates the H2A.X DNA damage response via a novel tyrosine kinase activity. *Nature*, 2009, **457**:57-62, doi:nature07668 [pii] 10.1038/nature07668

183. Singh, R. K., Kabbaj, M. H., Paik, J., Gunjan, A. Histone levels are regulated by phosphorylation and ubiquitylation-dependent proteolysis. *Nat Cell Biol*, 2009, **11**:925-933, doi:ncb1903 [pii] 10.1038/ncb1903

184. Dawson, M. A., Bannister, A. J., Gottgens, B., Foster, S. D., Bartke, T., Green, A. R., et al. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature*, 2009, **461**:819-822, doi:nature08448 [pii] 10.1038/nature08448

185. Mahajan, K., Fang, B., Koomen, J. M., Mahajan, N. P. H2B Tyr37 phosphorylation suppresses expression of replication-dependent core histone genes. *Nat Struct Mol Biol*, 2012, **19**:930-937, doi:10.1038/nsmb.2356

186. Wang, S. C., Nakajima, Y., Yu, Y. L., Xia, W., Chen, C. T., Yang, C. C., et al. Tyrosine phosphorylation controls PCNA function through protein stability. *Nat Cell Biol*, 2006, **8**:1359-1368, doi:10.1038/ncb1501

187. Huo, L., Wang, Y. N., Xia, W., Hsu, S. C., Lai, C. C., Li, L. Y., et al. RNA helicase A is a DNA-binding partner for EGFR-mediated transcriptional activation in the nucleus. *Proc Natl Acad Sci U S A*, 2010, **107**:16125-16130, doi:10.1073/pnas.1000743107

188. Yu, Y. L., Chou, R. H., Wu, C. H., Wang, Y. N., Chang, W. J., Tseng, Y. J., et al. Nuclear EGFR suppresses ribonuclease activity of polynucleotide phosphorylase through DNAPK-mediated phosphorylation at serine 776. *J Biol Chem*, 2012, **287**:31015-31026, doi:10.1074/jbc.M112.358077

189. Zhao, H., Lo, Y. H., Ma, L., Waltz, S. E., Gray, J. K., Hung, M. C., et al. Targeting tyrosine phosphorylation of PCNA inhibits prostate cancer growth. *Mol Cancer Ther*, 2011, **10**:29-36, doi:10.1158/1535-7163.MCT-10-0778

190. Yu, Y. L., Chou, R. H., Liang, J. H., Chang, W. J., Su, K. J.,

Tseng, Y. J., et al. Targeting the EGFR/PCNA signaling suppresses tumor growth of triple-negative breast cancer cells with cell-penetrating PCNA peptides. *PLoS One*, 2013, **8**:e61362, doi:10.1371/journal.pone.0061362

191. Reyes, D., Ballare, C., Castellano, G., Soronellas, D., Bago, J. R., Blanco, J., et al. Activation of mitogen- and stress-activated kinase 1 is required for proliferation of breast cancer cells in response to estrogens or progestins. *Oncogene*, 2014, **33**:1570-1580, doi:10.1038/onc.2013.95

192. Li, B., Wan, Z., Huang, G., Huang, Z., Zhang, X., Liao, D., et al. Mitogen- and stress-activated Kinase 1 mediates Epstein-Barr virus latent membrane protein 1-promoted cell transformation in nasopharyngeal carcinoma through its induction of Fra-1 and c-Jun genes. *BMC Cancer*, 2015, **15**:390, doi:10.1186/s12885-015-1398-3

193. Naqvi, S., Macdonald, A., McCoy, C. E., Darragh, J., Reith, A. D., Arthur, J. S. Characterization of the cellular action of the MSK inhibitor SB-747651A. *Biochem J*, 2012, **441**:347-357, doi:10.1042/BJ20110970

194. Johnson, J., Shi, Z., Liu, Y., Stack, M. S. Inhibitors of NF-kappaB reverse cellular invasion and target gene upregulation in an experimental model of aggressive oral squamous cell carcinoma. *Oral Oncol*, 2014, **50**:468-477, doi:10.1016/j.oraloncology.2014.02.004

195. Pardanani, A. JAK2 inhibitor therapy in myeloproliferative disorders: rationale, preclinical studies and ongoing clinical trials. *Leukemia*, 2008, **22**:23-30, doi:10.1038/sj.leu.2404948

196. Wu, S. C., Li, L. S., Kopp, N., Montero, J., Chapuy, B., Yoda, A., et al. Activity of the Type II JAK2 Inhibitor CHZ868 in B Cell Acute Lymphoblastic Leukemia. *Cancer Cell*, 2015, **28**:29-41, doi:10.1016/j.ccell.2015.06.005

197. Mueller, S., Haas-Kogan, D. A. WEE1 Kinase As a Target for Cancer Therapy. *J Clin Oncol*, 2015, doi:10.1200/JCO.2015.62.2290

198. Li, J., Wang, Y., Sun, Y., Lawrence, T. S. Wild-type TP53 inhibits G(2)-phase checkpoint abrogation and radiosensitization induced by PD0166285, a WEE1 kinase inhibitor. *Radiat Res*, 2002, **157**:322-330,

199. Hashimoto, O., Shinkawa, M., Torimura, T., Nakamura, T., Selvendiran, K., Sakamoto, M., et al. Cell cycle regulation by the Wee1 inhibitor PD0166285, pyrido [2,3-d] pyimidine, in the B16 mouse melanoma cell line. *BMC Cancer*, 2006, **6**:292, doi:10.1186/1471-2407-6-292

200. Hirai, H., Arai, T., Okada, M., Nishibata, T., Kobayashi, M., Sakai, N., et al. MK-1775, a small molecule Wee1 inhibitor, enhances anti-tumor efficacy of various DNA-damaging agents, including 5-fluorouracil. *Cancer Biol Ther*, 2010, **9**:514-522,

201. Bridges, K. A., Hirai, H., Buser, C. A., Brooks, C., Liu, H., Buchholz, T. A., et al. MK-1775, a novel Wee1 kinase inhibitor, radiosensitizes p53-defective human tumor cells. *Clin Cancer Res*, 2011, **17**:5638-5648, doi:10.1158/1078-0432.CCR-11-0650

202. Rajeshkumar, N. V., De Oliveira, E., Ottenhof, N., Watters, J., Brooks, D., Demuth, T., et al. MK-1775, a potent Wee1 inhibitor, synergizes with gemcitabine to achieve tumor regressions, selectively in p53-deficient pancreatic cancer xenografts. *Clin Cancer Res*, 2011, **17**:2799-2806, doi:10.1158/1078-0432.CCR-10-2580

203. Kreahling, J. M., Gemmer, J. Y., Reed, D., Letson, D., Bui, M., Altiok, S. MK1775, a selective Wee1 inhibitor, shows single-agent antitumor activity against sarcoma cells. *Mol Cancer Ther*, 2012, **11**:174-182, doi:10.1158/1535-7163.MCT-11-0529

204. Guertin, A. D., Li, J., Liu, Y., Hurd, M. S., Schuller, A. G., Long, B., et al. Preclinical evaluation of the WEE1 inhibitor MK-1775 as single-agent anticancer therapy. *Mol Cancer Ther*, 2013, **12**:1442-1452, doi:10.1158/1535-7163.MCT-13-0025

205. Qi, W., Xie, C., Li, C., Caldwell, J. T., Edwards, H., Taub, J. W., et al. CHK1 plays a critical role in the anti-leukemic activity of the wee1 inhibitor MK-1775 in acute myeloid leukemia cells. *J Hematol Oncol*, 2014, **7**:53, doi:10.1186/s13045-014-0053-9

206. Do, K., Wilsker, D., Ji, J., Zlott, J., Freshwater, T., Kinders, R. J., et al. Phase I Study of Single-Agent AZD1775 (MK-1775), a Wee1 Kinase Inhibitor, in Patients With Refractory Solid Tumors. *J Clin Oncol*, 2015, doi:10.1200/JCO.2014.60.4009

207. Pokorny, J. L., Calligaris, D., Gupta, S. K., Iyekegbe, D. O., Jr., Mueller, D., Bakken, K. K., et al. The Efficacy of the Wee1 Inhibitor MK-1775 Combined with Temozolomide Is Limited by Heterogeneous Distribution across the Blood-Brain Barrier in Glioblastoma. *Clin Cancer Res*, 2015, **21**:1916-1924, doi:10.1158/1078-0432.CCR-14-2588

208. Zhou, L., Zhang, Y., Chen, S., Kmieciak, M., Leng, Y., Lin, H., et al. A regimen combining the Wee1 inhibitor AZD1775 with HDAC inhibitors targets human acute myeloid leukemia cells harboring various genetic mutations. *Leukemia*, 2015, **29**:807-818, doi:10.1038/leu.2014.296

209. Chen, B., Duan, L., Yin, G., Tan, J., Jiang, X. miR-381, a novel intrinsic WEE1 inhibitor, sensitizes renal cancer cells to 5-FU by up-regulation of Cdc2 activities in 786-O. *J Chemother*, 2013, **25**:229-238, doi:10.1179/1973947813Y.0000000092

210. Nielsen, J. S., Jakobsen, E., Holund, B., Bertelsen, K., Jakobsen, A. Prognostic significance of p53, Her-2, and EGFR overexpression in borderline and epithelial ovarian cancer. *Int J Gynecol Cancer*, 2004, **14**:1086-1096, doi:10.1111/j.1048-891X.2004.14606.x

211. Dimri, M., Naramura, M., Duan, L., Chen, J., Ortega-Cava, C., Chen, G., et al. Modeling breast cancer-associated c-Src and EGFR overexpression in human MECs: c-Src and EGFR cooperatively promote aberrant three-dimensional acinar structure and invasive behavior. *Cancer Res*, 2007, **67**:4164-4172, doi:10.1158/0008-5472.CAN-06-2580

212. Nieto, Y., Nawaz, F., Jones, R. B., Shpall, E. J., Nawaz, S. Prognostic significance of overexpression and phosphorylation of epidermal growth factor receptor (EGFR) and the presence of truncated EGFRvIII in locoregionally advanced breast cancer. *J Clin Oncol*, 2007, **25**:4405-4413, doi:10.1200/JCO.2006.09.8822

213. Kong, J. H., Park, Y. H., Kim, J. A., Kim, J. H., Yun, J., Sun, J. M., et al. Patterns of skin and soft tissue metastases from breast cancer according to subtypes: relationship between EGFR overexpression and skin manifestations. *Oncology*, 2011, **81**:55-62, doi:10.1159/000331417

214. Kallel, I., Khabir, A., Boujelbene, N., Abdennadher, R., Daoud, J., Frikha, M., et al. EGFR overexpression relates to triple negative profile and poor prognosis in breast cancer patients in Tunisia. *J Recept Signal Transduct Res*, 2012, **32**:142-149, doi:10.3109/10799893.2012.664552

215. Tang, Y., Zhu, L., Li, Y., Ji, J., Li, J., Yuan, F., et al. Overexpression of epithelial growth factor receptor (EGFR) predicts better response to neo-adjuvant chemotherapy in patients with triple-negative breast cancer. *J Transl Med*, 2012, **10 Suppl 1**:S4, doi:10.1186/1479-5876-10-S1-S4

216. Angulo, B., Suarez-Gauthier, A., Lopez-Rios, F., Medina, P. P., Conde, E., Tang, M., et al. Expression signatures in lung cancer reveal a profile for EGFR-mutant tumours and identify selective PIK3CA overexpression by gene amplification. *J Pathol*, 2008, **214**:347-356, doi:10.1002/path.2267

217. Hirsch, F. R., Varella-Garcia, M., Cappuzzo, F. Predictive value of EGFR and HER2 overexpression in advanced non-small-cell lung cancer. *Oncogene*, 2009, **28 Suppl 1**:S32-37, doi:10.1038/onc.2009.199

218. Chen, C. C., Chiu, H. H., Yen, L. C., Chang, H. J., Chang, M. S., Tsai, J. R., et al. Overexpression of EGFR pathway-related genes in the circulation is highly correlated with EGFR mutations and overexpression in paired cancer tissue from patients with non-small cell lung cancer. *Oncol Rep*, 2010, **23**:639-645,

219. Sabattini, S., Mancini, F. R., Marconato, L., Bacci, B., Rossi, F., Vignoli, M., et al. EGFR overexpression in canine primary lung cancer: pathogenetic implications and impact on survival. *Vet Comp Oncol*, 2014, **12**:237-248, doi:10.1111/vco.12002

220. Guo, G. F., Cai, Y. C., Zhang, B., Xu, R. H., Qiu, H. J., Xia, L. P., et al. Overexpression of SGLT1 and EGFR in colorectal cancer showing a correlation with the prognosis. *Med Oncol*, 2011, **28 Suppl 1**:S197-203, doi:10.1007/s12032-010-9696-8

221. Bulgaru, A. M., Mani, S., Goel, S., Perez-Soler, R. Erlotinib (Tarceva): a promising drug targeting epidermal growth factor receptor tyrosine kinase. *Expert Rev Anticancer Ther*, 2003, **3**:269-279, doi:10.1586/14737140.3.3.269

222. Yano, S., Yamaguchi, M., Dong, R. P. EGFR tyrosine kinase inhibitor «gefitinib (Iressa)» for cancer therapy. *Nihon Yakurigaku Zasshi*, 2003, **122**:491-497,

223. Ng, M., Cunningham, D. Cetuximab (Erbitux)--an emerging targeted therapy for epidermal growth factor receptor-expressing tumours. *Int J Clin Pract*, 2004, **58**:970-976,

224. Johnston, S. R., Leary, A. Lapatinib: a novel EGFR/HER2 tyrosine kinase inhibitor for cancer. *Drugs Today (Barc)*, 2006, **42**:441-453, doi:10.1358/dot.2006.42.7.985637

225. Chen, Y. J., Yeh, M. H., Yu, M. C., Wei, Y. L., Chen, W. S., Chen, J. Y., et al. Lapatinib-induced NF-kappaB activation sensitizes triple-negative breast cancer cells to proteasome inhibitors. *Breast Cancer Res*, 2013, **15**:R108, doi:10.1186/bcr3575

226. Hsia, T. C., Tu, C. Y., Chen, Y. J., Wei, Y. L., Yu, M. C., Hsu, S. C., et al. Lapatinib-mediated cyclooxygenase-2 expression via epidermal growth factor receptor/HuR interaction enhances the aggressiveness of triple-negative breast cancer cells. *Mol Pharmacol*, 2013, **83**:857-869, doi:10.1124/mol.112.082743

227. Cohenuram, M., Saif, M. W. Panitumumab the first fully human monoclonal antibody: from the bench to the clinic. *Anticancer Drugs*, 2007, **18**:7-15, doi:10.1097/CAD.0b013e32800feecb

228. Li, Y., Ahmad, A., Sarkar and F. H., ASPP and iASPP: Implication in cancer development and progression. *Cell. Mol. Biol.* 2015, **61(6)**: 2-8.

229. Masood, N.,, Qureshi, M. Z. and Yasmin, A., Association of NOTCH with different microRNAs in head and neck cancer. *Cell. Mol. Biol.* 2015, **61(6)**: 9-16.

230. Amirkhah, R., Farazmand, A., Irfan-Maqsood, M., Wolkenhauerand, O. and Schmitz, U., The role of microRNAs in the resistance to colorectal cancer treatments. *Cell. Mol. Biol.* 2015, **61(6)**: 17-23.

231. Wang, Z., Chen, J. and Capobianco, A. J., The Notch signaling pathway in esophageal adenocarcinoma. *Cell. Mol. Biol.* 2015, **61(6)**: 24-32.

232. Limami, Y., Pinon, A., Riaz, A. and Simon, A., TRAIL and targeting cancer cells: between promises and obstacles. *Cell. Mol. Biol.* 2015, **61(6)**: 33-38.

233. Silva Galbiatti-Dias, A. L., Pavarino, É. C., Kawasaki-Oyama, R. S., Maniglia, J. V., Maniglia, E. J. V. and Goloni Bertollo, E. M., Cancer stem cells in head and neck cancer: A Mini Review. *Cell. Mol. Biol.* 2015, **61(6)**: 39-43.

234. Musella, A., Marchetti, C., Gasparri, M. L., Salerno, L., Casorelli, A., Domenici, L., Imperiale, L., Ruscito, I., Abdul Halim, T., Palaia, I., Di Donato, V., Pecorini, F., Monti, M., Muzii, L. and Panici, P. B., PARP inhibition: A promising therapeutic target in ovarian cancer. *Cell. Mol. Biol.* 2015, **61(6)**: 44-61.

235. Attar, R., Tabassum, S., Fayyaz, S., Ahmad, M. S., Nogueira, D. R., Yaylim, I., Timirci-Kahraman, O., Kucukhuseyin, O., Cacina, C., Farooqi, A. A. and Ismail, M., Natural products are the future of anticancer therapy: Preclinical and clinical advancements of *Viscum album* phytometabolites. *Cell. Mol. Biol.* 2015, **61(6)**: 62-68.

236. Chong, L-W., Chou, R-H., Liao, C-C., Lee, T-F., Lin, Y., Yang, K-C. and Hsu, Y-C. Saturated fatty acid induces cancer stem cell-like properties in human hepatoma cells. *Cell. Mol. Biol.* 2015, **61(6)**: 85-91.

237. Smina, T. P., Mohan, A., Ayyappa, K. A., Sethuraman, S. and Krishnan, U. M., Hesperetin exerts apoptotic effect on A431 skin carcinoma cells by regulating mitogen activated protein kinases and cyclins. *Cell. Mol. Biol.* 2015, **61(6)**: 92-99.

238. Ahmadi, M., Jafari, R., Marashi, S. A. and Farazmand, A., Indirect role of microRNAs and transcription factors in the regulation of important cancer genes: A network biology approach. *Cell. Mol. Biol.* 2015, **61(6)**: 100-107.

239. Zahoor, A., Mansoor, Q., Farooqi, A. A., Fayyaz, S., Naz, G. and Ismail, M., Genetic variants in the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and death receptor (DR4) genes contribute to susceptibility to colorectal cancer in pakistani population. *Cell. Mol. Biol.* 2015, **61(6)**: 108-112.