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Aberrant histone modification in endometriosis

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1. ABSTRACT

Accumulating evidence suggests that epigenetic aberrations play definite roles in the pathogenesis of endometriosis. These include aberrations in genomic DNA methylation, microRNA expression, and histone modification. The aberrant histone modification status and the aberrant expression of histone deacetylases, which regulate histone acetylation, in endometriosis are the focus of this review. Herein, we summarize the recent studies in the following areas: (i) hyperacetylation of histones located in the promoter lesions of G-protein-coupled estrogen receptor 1, steroidogenic factor-1, and hypoxia-inducible factor-1 alpha genes and (ii) hypoacetylation of histones located in the promoter lesions of estrogen receptor alpha, homeobox A10, CCAAT/enhancer-binding protein alpha, $p16^{INK4a}$, $p21^{Waf1/Cip1}$, $p27^{Kip1}$, checkpoint kinase 2, death receptor 6, and E-cadherin genes. Further research from the viewpoint of epigenetics may lead to the identification of the candidate molecules that are aberrantly expressed in endometriosis and may help elucidate the pathogenesis of this disease. In addition, epigenetic drugs (including histone deacetylase inhibitors) show promise for the treatment of endometriosis by amending the expression of these epigenetically dysregulated genes.

2. INTRODUCTION

Histological evaluations have revealed that endometriotic tissues share many characteristics with normal proliferative endometrial tissues (1), but they also show several molecular differences. These include differences in gene expression and protein production, production of and responsiveness to steroids and cytokines, proteolytic enzymes and their inhibitors, adhesion molecules, immune components, and tissue structure and cell proliferation rate (2). Together, aberrations in these molecular processes may account for the occurrence and development of endometriosis. Such aberrant expressions are seemingly quite stable and consistent in endometriosis, suggesting the involvement of some sort of cellular memory.

Several groups have observed constitutive aberrations in endometriosis, such as genomic alterations and the loss of heterozygosity (3–6). However, no genes linked to these events have been identified. Other possible contributors to endometriosis are alterations in the abundance of transcripts and post-transcriptional regulatory mechanisms. Inheritance of the phenotypes of cells and entire organisms without any alterations in their DNA

content or DNA sequence is the basis of epigenetics (7, 8). The epigenetic information of any organism, animal, or human being is transferred to the next generation in one of two ways: at the cellular level or at the whole-organism level. The epigenetic information is not encoded into a DNA sequence. Rather, the phenotypes are established by nuclear processes (e.g., chromatin modifications such as the methylation, acetylation, ubiquitination, phosphorylation, biotinylation, sumoylation, ribosylation, and isomerization of histones; and DNA methylation) and by microRNA and double-stranded noncoding RNA, which are interconnected and may work together to establish and maintain specific gene activity states in normal cells (7, 9–11).

Among the many epigenetic modification mechanisms that exist, histone acetylation is one of the best-understood. Histone acetylation levels are controlled by a balance between the actions of histone deacetylases (HDACs) and histone acetyltransferases. The latter of these two enzymes transfers acetyl groups from acetyl-coenzyme A to lysine residues on the N-terminal region of the histones to activate genetic transcription. HDACs, on the other hand, are large multiprotein complexes that target promoter sites through their interaction with the sequence-specific transcription process (12). They remove the acetyl groups, restoring the positive charge on lysine residues and thus preventing transcription. HDAC inhibitors (HDACIs) show antiproliferative activity, induce cell-cycle arrest, and stimulate apoptosis (13).

It is becoming apparent that several histone modification mechanisms have roles in the pathogenesis of endometriosis (14–17), and it was demonstrated that HDACIs may be effective for the treatment of this disease (14, 18–20). This review summarizes the recent findings on the aberrant histone modification status and the aberrant expression of histone deacetylases that regulate histone acetylation in endometriosis. Most of the epigenetically dysregulated genes are suggested to be responsible for the proliferative, anti-apoptotic, and other disease-specific characteristics of endometriosis, thereby contributing to the pathogenesis of the disease. We also discuss the therapeutic potentials of epigenetic drugs, including histone deacetylase inhibitors, for the treatment and prevention of endometriosis.

3. GLOBAL HISTONE ACETYLATION AND METHYLATION STATUS IN ENDOMETRIOSIS

Although aberrant histone modifications are known to play a role in the pathogenesis of endometriosis, the histone modification profile of endometriotic tissues has not been established. In our comparison of eutopic endometrial stromal cells from unaffected women and ovarian endometriotic stromal cells, we found the acetylated levels of histones H3 and H4 to be significantly lower in the latter type of cells (14). It is of note that H3, but not H4, is globally hypoacetylated in endometriosis, as reported by Monteiro *et al*. (21). Their study also revealed significantly lower levels of H3K9ac and H4K16ac in this disease. Xiaomeng *et al*. (22) evaluated the global H3/H4 acetylation in endometriotic lesions and in the eutopic endometrium of patients with endometriosis and found H4 to be hypoacetylated in both tissue types compared with the eutopic endometrium of unaffected women.

In the same study, and using the same tissue types for comparison, Xiaomeng *et al*. (22) evaluated the global H3K4/H3K9 methylation status. They observed global hypomethylation of H3K4 and of H3K9 in both endometriotic lesions and the eutopic endometrium of patients with endometriosis. Similarly, Monteiro *et al*. (21) demonstrated that H3K4, H3K9, and H3K27 were hypermethylated in endometriotic lesions. These recent data suggest that aberrant genome-wide histone modifications occur in endometriosis, which would result in the dysregulated expression of endometriosis-associated genes.

4. HDAC EXPRESSION IN ENDOMETRIOSIS

HDACs covalently deacetylate key residues in histones, thereby altering the conformation of chromatin, which results in transcriptional repression (23, 24). Histone deacetylation generally results in gene silencing, and thus HDACs act as transcriptional repressors. Munro *et al*. (25) found that the global histone acetylation in the eutopic endometrium of unaffected women increases when transcription activity is likely to be higher, suggesting that histone acetylation/deacetylation is hormonally regulated and that abnormal epigenetic modifications may be associated with endometriosis. Thus, the HDAC isoforms that are involved should be identified, and the mechanisms that are activated in the endometriotic lesions via HDAC expression and function must be explored. However, the complete HDAC expression profile of endometriotic lesions has not yet been determined.

Of the 18 HDACs identified to date, HDAC1 and HDAC2 are conserved enzymes that are part of the Sin3a repressor complex (26). Sin3a transcriptionally regulates the genes encoding the Snail-mediated E-cadherin repression, glucocorticoid receptor-mediated transcription, and steroidogenic acute regulatory proteins (27, 28). HDAC1 and HDAC2 show overlapping functions in the regulation of the cell cycle, and their expressions were observed to correlate with the levels of Ki-67, a nuclear protein associated with cellular proliferation (29, 30). The silencing of HDAC1 and HDAC2 is followed by cell-cycle arrest and the upregulation of the cyclin-dependent kinase inhibitor $p21^{Waf1/Cip1}$ (31).

Throughout the menstrual cycle, the eutopic, cycling endometrium constitutively expresses Class I HDACs. The HDAC2 protein was found to be slightly elevated during the secretory phase (32). The basal and hormone-regulated gene expression levels of HDAC1 and HDAC2 in endometriotic cells were evaluated by Colón-Díaz *et al*. (33), as were the protein expression levels in tissues. The basal HDAC1 and HDAC2 gene expression levels were significantly higher in the endometriotic stromal cells than in the eutopic endometrial stromal cells of patients with endometriosis. In addition, in eutopic endometrial epithelial cells of affected patients, the

Table 1. Histone modification associated aberrant gene expression in endometriosis

Gene name	Histone modification status	Effect of HDACI on gene expression	Aberrant gene espression	Tissues and cells	References
ERα	Hypoacetylated	N.D.	Downregulated	Endometriotic tissues and endometriotic stromal cells	32
GPER1	N.D.	Downregulation	Upregulated	Endometriotic tissue	54
SF-1	Hyperacetylated	N.D.	Upregulated	Endometriotic stromal cells	32
HOXA10	Hypoacetylated	N.D.	Downregulated	Endometriotic tissue and eutopic endometrium of patients with endometriosis	32
C/EBPα	Hypoacetylated	Upregulation	Downregulated	Endometriotic tissue, eutopic endometrium of patients with endometriosis, and endometriotic stromal cells	28
HIF-1α	N.D.	Downregulation	Upregulated	Endometriotic tissue	68
DR6	Hypoacetylated	Upregulation	Downregulated	Endometriotic tissue, eutopic endometrium of patients with endometriosis, and endometriotic stromal cells	113
E-cadherin	Hypoacetylated	Upregulation	Downregulated	Endometriotic cells	32, 117, 118

C/EBPα, CCAAT/enhancer-binding protein α; Chek2, Checkpoint kinase 2; DR6, death receptor 6; ERα, estrogen receptor α; GPER1, G-protein-coupled estrogen receptor 1; HIF-1α, hypoxia-inducible factor-1α; HOXA10, homeobox A10; N.D., not described; SF-1, steroidogenic factor-1.

HDAC1 expression was significantly downregulated by estradiol and progesterone. Moreover, in eutopic endometrial stromal cells of unaffected women, the levels of HDAC2 were upregulated by estradiol and downregulated by a combination of estradiol and progesterone. In the endometriotic cells, the hormone modulation of HDAC1 and HDAC2 gene expression was lost.

When Samartzis *et al*. (34) used a tissue array to assess the expressions of the Class I histone deacetylases (HDAC1, HDAC2, and HDAC3) in endometriotic lesions and in the eutopic endometrium from women with and without endometriosis, they observed the expression of HDAC1 to be significantly higher in the endometriotic tissues, whereas the expressions of HDAC2 and HDAC3 were similar in the two types of eutopic endometrial tissues. Significant correlations between HDAC1 and estrogen receptor (ER) α/ERβ and progesterone receptor expression were also observed. However, Xiaomeng *et al*. (22) demonstrated the mRNA levels of HDAC1 to be significantly downregulated in endometriotic lesions, and the HDAC2 mRNA level to be significantly increased in the eutopic endometrium of patients with endometriosis. They also showed the level of SIRT1 mRNA to be significantly decreased in the eutopic endometrium of affected patients. The discrepancies of these findings may be due to the differences of experimental methods used in each study.

5. HISTONE MODIFICATIONS IN ENDOMETRIOSIS-ASSOCIATED GENES

As shown in Table 1, aberrant histone acetylations of endometriosis-associated genes have been reported in the literature.

5.1. ERα

The estrogen receptors ERα and ERβ, which act as transcription factors, are thought to have key roles in regulating the growth of the endometrium and endometriosis (35, 36). Markedly higher levels of ERβ and lower levels of ERα have been observed in endometriotic tissues and in endometriotic stromal cells (35–37). The differences in the ERα/ERβ ratio between these two cell types could have important functional implications, since these ERs have different ligand-binding characteristics (38, 39). Using chromatin immunoprecipitation (ChIP) analysis, Monteiro *et al*. (21) recently showed the hypoacetylation of H3/H4 within the promoter regions of the ERα gene in endometriotic lesions.

5.2. G-protein-coupled estrogen receptor 1

G-protein-coupled estrogen receptor 1 (GPER1) is a novel estrogen receptor that mediates both rapid events and transcriptional events in response to estrogen. The GPER1-mediated biological responses to estrogen involve multiple physiological intracellular processes, including the activation of signaling pathways (40, 41).

Samartzis *et al*. (42) recently showed the GPER1 expression level in endometriotic lesions to be higher than that in the eutopic endometrium of patients with endometriosis. GPER1 was also found to be upregulated in ovarian endometriotic lesions compared with normal human ovarian tissue (43). Imesch *et al*. (44) demonstrated that GPER1 plays an important role in mediating endometriosis-derived 11z epithelial cell proliferation and signal transduction. It was proposed that GPER1 could be a potential therapeutic target of endometriosis through the reduction of its estrogen dependence (42). The functional inhibition of GPER1 by its antagonist decreases the proliferation of endometriotic cells (44). The HDACIs romidepsin and SAHA both reduce GPER1 expression,

which is correlated with the accumulation of acetylated histones (44).

5.3. Nuclear receptor subfamily 5, group A, member 1 (steroidogenic factor-1)

The transcriptional factor nuclear receptor subfamily 5, group A, member 1, also known as steroidogenic factor-1 (SF-1), is essential for the activation of multiple steroidogenic genes for estrogen biosynthesis. These genes include the ones coding for the steroidogenic acute regulatory protein (STAR) and the cytochrome P450, family 19, subfamily A, polypeptide 1 (CYP19A1) protein (45–47). SF-1 is usually undetectable in eutopic endometrial stromal cells of unaffected women (48). The SF-1 mRNA and protein levels in endometriotic stromal cells were shown to be significantly higher than those in eutopic endometrial stromal cells of women without endometriosis (47, 48). Xue *et al*. (48) identified a classical CpG island at the promoter region of the SF-1 gene and showed the methylation of the SF-1 promoter to be increased in eutopic endometrial cells of unaffected women. Monteiro *et al*. (21) demonstrated the SF-1 promoter region to be also enriched for acetylated H3 and H4 in endometriotic lesions, correlating with its reported high expression in those lesions. The epigenetically induced SF-1 expression in endometriosis may enhance the CYP19A1 expression and local estrogen production.

5.4. Homeobox A10

Homeobox A10 **(**HOXA10) is a member of the homeobox gene family, all members of which contain a common conserved region of 183 basepairs called a homeobox (49). The homeobox gene family serves as transcription factors during development and has been shown to be important for uterine function (50). HOXA10 was shown to be expressed in the eutopic endometrium, and to be regulated by estrogen and progesterone (50–52). Its peak expression occurs during the window of implantation, suggesting possible roles in endometrial development during the menstrual cycle and in establishing uterine receptivity (50, 51).

Taylor *et al*. (51) demonstrated the expression of HOXA10 to be significantly reduced in the eutopic endometrium of patients with endometriosis during the secretory phase, indicating the presence of some functional defects in uterine receptivity. Those authors also observed a decreased expression of HOXA10 in endometriotic lesions. Wu *et al*. (53) reported that the promoter region of the HOXA10 gene was hypermethylated in the eutopic endometrium of women with endometriosis compared with unaffected women. As promoter hypermethylation is generally associated with gene silencing, the observed HOXA10 promoter hypermethylation provides a plausible explanation for the reduced expression of HOXA10 in the eutopic endometrium of women with endometriosis (51). In endometriosis, the altered expression of the progesterone receptor or its diminished activity may lead to an attenuated response to progesterone and the decreased expression of progesterone-responsive genes, including the HOXA10 gene, in the eutopic endometrium. The long-term suppression of HOXA10 expression in endometriosis may further induce the epigenetic alteration by hypermethylation at the promoter region.

Recently, using ChIP analysis, Monteiro *et al*. (21) showed the hypoacetylation of H3/H4 within the promoter regions of the HOXA10 gene in endometriotic lesions.

5.5. CCAAT/enhancer-binding protein α

Using the mRNA microarray technique, we found five HDAC1-target mRNAs that were upregulated in endometriotic stromal cells after treatment with valproic acid (VPA) (17). Of these candidate mRNAs, we focused on CCAAT/enhancer-binding protein (C/EBP) α and designed detailed observational and functional experiments to study this transcription factor.

C/EBPα is a member of the C/EBP family of transcription factors that play a role in a variety of physiological processes, including cell-cycle control, cell proliferation, differentiation, metabolism, and inflammation (54). C/EBPα consists of an N-terminal transcriptional activation domain and a C-terminal basic leucine zipper (bZIP), and it forms a homo- or heterodimer with C/EBP family proteins or other transcription factors to bind a set of related DNA recognition sequences. Yang *et al*. (55) demonstrated by immunohistochemistry that C/EBPα protein expression in the eutopic endometrium of patients with endometriosis tended to be higher than that in endometriotic lesions.

We found the expressions of C/EBPα mRNA and protein to be attenuated in the endometriotic stromal cells, and upregulated by VPA stimulation (17). VPA treatment significantly inhibited the HDAC activity and resulted in the accumulation of acetylated H3 and H4 in the promoter region of the C/EBPα gene in endometriotic stromal cells. The compulsory expression of C/EBPα in these cells directed the inhibition of their proliferation and the induction of their apoptosis, whereas C/EBPα knockdown by siRNA in eutopic endometrial stromal cells of unaffected women directed the stimulation of cell proliferation and their resistance to apoptosis. The following target genes of C/EBPα are thought to be involved in these phenomena: apoptosis-inducing factor, mitochondrion-associated, 1 (AIFM1); BCL2-associated X protein (Bax); caspase-10; caspase-8; cyclin-dependent kinase (CDK) 2 and CDK4; CDK inhibitor 2A ($p16^{INK4a}$); CDK inhibitor 1A ($p21^{Waf1/Cip1}$); tumor protein p53; period circadian clock 2 (PER2); and peroxisome proliferator-activated receptor (PPAR)-γ.

5.6. Hypoxia-inducible factor-1α

The transcription hypoxia-inducible factor-1α (HIF-1α) is induced by hypoxic conditions, which promote angiogenesis. Compared with eutopic endometrial tissue, endometriotic tissue showed higher levels of HIF-1α mRNA and protein (56), a finding that highlights the roles of HIF-1α and hypoxia in endometriosis. HIF-1α is also subject to post-translational modifications; for example, although under normal conditions HIF-1α is hydroxylated,

acetylated, and subject to rapid polyubiquitin-dependent proteosomal degradation, under hypoxic conditions, it is stabilized via dehydroxylation and deacetylation. When stabilized, HIF-1α heterodimerizes with HIF-1β, and the heterodimer binds to the vascular endothelial growth factor (VEGF) promoter to activate its transcription (57, 58). HIF-1α stabilization is thought to involve histone deacetylases, in particular HDAC1 and HDAC3.

The HDACI romidepsin downregulates the expressions of VEGF mRNA and protein in endometriotic cells, through the suppression of HIF-1α (59). Imesch *et al*. (60) recently demonstrated that romidepsin inhibits the proliferation of and activates apoptosis in endometriotic epithelial cells.

5.7. CDK inhibitor 2A ($p16^{INK4a}$), CDK inhibitor 1A ($p21^{Waf1/Cip1}$), and CDK inhibitor 1B ($p27^{Kip1}$)

The cell cycle is the series of events that leads to cell division and duplication, producing two daughter cells. CDKs are a family of protein kinases that regulate the cell cycle by binding the regulatory protein cyclin.

$p16^{INK4a}$ is a CDK inhibitor that controls cell-cycle progression during the G1 phase by inhibiting the ability of cyclin D–CDK4 and cyclin D–CDK6 complexes to phosphorylate retinoblastoma 1 (RB1) (61, 62). RB1 remains associated with E2F transcription factor 1 (E2F1), localizing it to the cytoplasm and thus preventing the transcription of E2F1 target genes that are crucial for the G1/S transition. We demonstrated that HDACIs induce $p16^{INK4a}$ expression by accumulating acetylated H3 and H4 in its promoter region, resulting in the suppression of cell proliferation, induction of cell-cycle arrest, and apoptosis of endometriotic stromal cells (14).

$p21^{Waf1/Cip1}$ is a CDK inhibitor that binds to cyclin–CDK complexes and decreases the kinase activity, and it may block cell-cycle progression at the G0/G1 phase as part of the tumor protein P53 pathway (63–65). $p21^{Waf1/Cip1}$-mediated growth inhibition has been attributed to two main activities that depend on two non-overlapping structural domains: the C-terminal proliferating cell nuclear antigen (PCNA)-binding domain and the N-terminal CDK–cyclin inhibitory domain (66, 67). By binding to PCNA, $p21^{Waf1/Cip1}$ prevents DNA polymerase-γ and several other proteins involved in DNA synthesis from binding with PCNA, thus directly inhibiting DNA synthesis (68). $p21^{Waf1/Cip1}$ also inhibits CDK activity indirectly by interfering with the activating phosphorylation of CDK1 and CDK2 in the activation segment (69–71).

HDACIs induce the expression of $p21^{Waf1/Cip1}$ by accumulating acetylated H3/H4 in its promoter region, likewise resulting in the suppression of cell proliferation, the induction of cell-cycle arrest, and the apoptosis of endometriotic stromal cells (14). Consistently, using ChIP analysis, Monteiro *et al*. (21) also showed the hypoacetylation of H3/H4 within promoter regions of the *CDKN1A* gene in endometriotic lesions.

$p27^{Kip1}$ is a member of the Cip/Kip family of CDK inhibitors that bind to cyclin–CDK complexes and decrease the kinase activity, and it may block cell-cycle progression from the G0/G1 phase to the S phase (65, 72–75). Various functions have been attributed to $p27^{Kip1}$, including the promotion of apoptosis (72, 76), the regulation of cell migration (75, 77), and the regulation of drug resistance (78). We demonstrated that HDACIs induce the expression of $p27^{Kip1}$ by accumulating acetylated H3/H4 in its promoter region, resulting in the same cellular events as noted above for endometriotic stromal cells (14).

5.8. Checkpoint kinase 2

Checkpoint kinase 2 (chek2) encodes a nuclear serine/threonine kinase that plays an integral role in the DNA damage response. It helps maintain genome integrity of by regulating the G2/M cell-cycle checkpoints, DNA repair, and apoptosis (79–81). chek2 is a stable, long-lived, constitutively expressed, and predominantly nuclear protein that is activated upon DNA damage in all phases of the mammalian cell cycle as part of the tumor protein P53 pathway (80, 82). It is activated mainly by serine-protein kinase ATM (A-T mutated) in response to double-stranded DNA breaks, and its activation involves dimerization and autophosphorylation (83, 84).

We demonstrated in endometriotic stromal cells that HDACIs induce the expression of chek2 by accumulating acetylated H3/H4 in its promoter region, producing the same cellular consequences that result from the accumulation of these acetylated histones (14).

5.9. Tumor necrosis factor receptor superfamily, member 21 (death receptor 6)

Cells undergo apoptosis via two major pathways: the extrinsic type I pathway (death receptor pathway) and the intrinsic type II pathway (the mitochondrial pathway). The type I pathway is mediated by the tumor necrosis factor receptor superfamily (TNFRSF), which includes TNFRSF1A (death receptor (DR) 1) (85), Fas cell surface DR (DR2, CD95, APO-1) (86), TNFRSF25 (DR3, Apo3, WSL-1, LARD, TRAMP) (87), TBFRSF10A (DR4, CD261, TNF-related apoptosis-inducing ligand receptor (TRAILR) 1) (88), TBFRSF10B (DR5, CD262, TRAILR2, TRICK2, KILLER) (89), TNFRSF21 (DR6) (90), ectodysplasin A (EDA) receptor, and the nerve growth factor receptor (NGFR). Upon ligation by their cognate ligands, these receptors engage a number of signal transduction pathways, including those involved in apoptosis, cellular survival, and stress response (91–95).

DR6 is distinguished by the presence of an alpha-helical structural motif called the death domain in the intracellular part (90, 96–98). It has been reported to be involved mostly in inflammatory responses, immune regulation (99–101), and neural cell death and maturation (102, 103). The cleaved-off N-terminal extracellular fragment of amyloid precursor protein and that of its close relative amyloid beta (A4) precursor-like protein 2 were found to function as DR6 ligands and to trigger DR6-dependent cell death (102).

Our microarray analysis comparing the gene expressions between VPA-treated and untreated endometriotic stromal cells revealed DR6 to be epigenetically suppressed in endometriosis through enhanced histone deacetylation (17). The expressions of DR6 mRNA and protein were significantly upregulated in endometriotic stromal cells after VPA treatment (104), whereas the expressions of other TNFRSFs (including those of DR1, DR2, DR3, DR4, DR5, DR6, EDAR, and NGFR) were not affected by the treatment. VPA treatment resulted in an accumulation of acetylated H4 in the promoter region of the DR6 gene. DR6 knockdown in eutopic endometrial stromal cells of unaffected women directed the stimulation of cell proliferation and the resistance to apoptosis.

The expressions of DR6 mRNA and protein in endometriotic stromal cells were significantly lower than those in eutopic endometrial stromal cells of unaffected women. DR6 expression in ovarian endometriotic lesions was also lower than that in the eutopic endometrium of affected and unaffected women (104).

5.10. Cadherin 1, type 1 (E-cadherin)

E-cadherin, transmembrane glycoprotein, is a member of the multigene superfamily of cadherins (105). It is a key molecule in the formation of adherence junctions and thus in the polarization and differentiation of intact epithelial tissue, such as endometrial glands (105). E-cadherin connects two neighboring cells via homophilic binding through their extracellular domains (105). Inhibition of E-cadherin expression and function is known to lead to the disruption of intercellular junctions, with a subsequent loss of cellular polarization, thus creating a honeycomb epithelial morphology that results in the induction of invasion and metastasis (106).

Endometriotic cells are found to lack E-cadherin expression, which is associated with the invasiveness of these cells (107). A ChIP analysis showed the hypoacetylation of H3/H4 within the promoter regions of the E-cadherin gene in endometriotic lesions (21). HDACIs were recently shown to reactivate E-cadherin expression, and attenuate the invasion and decrease the proliferation of endometriotic cells (108, 109).

6. EPIGENETIC DRUGS TARGETING HISTONE MODIFICATIONS

Endometriosis is treated in a number of ways, including by surgical interventions. The growth of endometriotic implants can be slowed by suppressing ovarian steroids and inducing a hypoestrogenic state, and the currently used treatments have been shown to be useful for relieving endometriosis-associated pain (110, 111). However, owing to their adverse effects, the endometriosis treatments that lower a patient's circulating estradiol concentrations cannot be administered indefinitely (112, 113). In addition, high recurrence rates of up to 45% after the completion of medical treatments remain a significant problem (114). New therapeutic strategies are necessary to improve the clinical management of patients with endometriosis.

As described above, epigenetic mechanisms represent a heritable, dynamic, and reversible means of modulating gene expression. Reversibility is an important characteristic of epigenetic aberrations, since it allows us to search for appropriate pharmacological treatments or "epigenetic therapies." A number of epigenetic therapies are currently under development, with the aim of potentially reversing the aberrant epigenetic events in affected cells. These approaches are directed toward modification of the DNA methylation profiles and histone modification states in target cells. They are based on the ability of specific properties of various chemical agents to affect the activity of enzymes involved in the establishment and maintenance of epigenetic marking. The target enzymes of epigenetic drugs include HDACs, histone acetyltransferases, DNA methyltransferases, histone methyltransferases, and histone demethylases (115). Among these, HDACIs are the most promising and most extensively studied as epigenetic agents. It is also noteworthy that SAHA, romidepsin, and VPA are already approved by the US Food and Drug Administration (FDA) for treating other diseases (116–118).

HDACIs inhibit cell proliferation and induce cell-cycle arrest and apoptosis by targeting the above-mentioned molecules in endometriotic cells *in vitro* (14, 18, 20, 44, 59, 60, 119). We showed that HDACIs, including VPA, SAHA, and apicidin, can inhibit the proliferation, induce cell differentiation and cell-cycle arrest, and stimulate apoptosis of endometriotic cells (14). Guo and his colleagues have demonstrated that HDACIs such as VPA and Trichostatin A can suppress the proliferation of endometriotic cells, as well as induce cell-cycle arrest, inhibit IL-1-beta-induced cyclooxygenase-2 expression and NF-κB activation, upregulate PPAR-γ, $p21^{Waf1/Cip1}$, and PR-B expression, attenuate invasiveness, and reactivate the silenced E-cadherin gene expression in these cells (18, 20, 108, 119–121). Romidepsin was also shown to specifically reduce HDAC enzymatic activity in endometriotic cells, resulting in inhibition of cell proliferation, cell-cycle arrest, increased apoptosis, and reduced expression of VEGF mRNA and protein in these cells (59, 60). Trichostatin and VPA were capable of reducing the lesion size and improving the response to hyperalgesia in murine and rat models of endometriosis (19, 122).

VPA was used in a pilot study of three patients with endometriosis and adenomyosis, who had moderate to severe dysmenorrhea (123). The patients were treated with a dose of 1,000 mg/day for 3 months. Complete relief from pain was reported in all cases, together with an average one-third reduction of the uterine size and the disappearance or reduction of palpable tender nodules in the cul-de-sac. Indeed, any beneficial effect of VPA must be confirmed by larger controlled studies.

Histone deacetylation is a potent regulator of gene expression in endometriosis, suggesting that HDACIs might be effective for the treatment of this disorder (14, 18). It should be noted that HDACIs lack specificity and therefore can lead to genome-wide inhibition of the deacetylation of histones, possibly increasing random gene

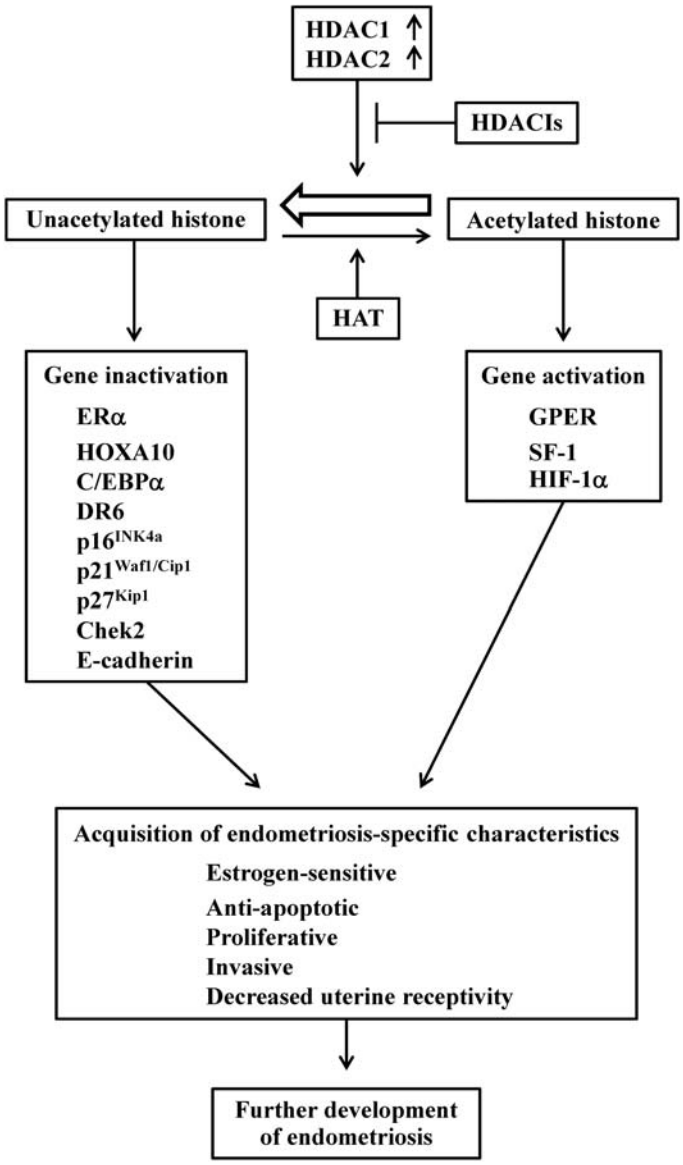

Figure 1. Possible roles of the aberrant histone modifications in the pathogenesis of endometriosis. HDAC1 and HDAC2 expressions have been reported to be upregulated in endometriosis. It has also been demonstrated that GPER, SF-1, and HIF-1α genes are activated by histone acetylation in endometriosis. Whereas, ERα, HOXA10, C/EBPα, DR6, $p16^{INK4a}$, $p21^{Waf1/Cip1}$, $p27^{Kip1}$, Chk2, and E-cadherin genes are inactivated by histone deacetylation in endometriosis. These aberrant histone modifications cause dysregulated gene expressions, resulting in the acquisition of endometriosis-specific characteristics and further development of endometriosis. HDACIs can reactivate the silenced gene transcription. C/EBPα, CCAAT/enhancer-binding protein α; chk2, cell cycle checkpoint kinase 2; DR6, death receptor 6; ERα, estrogen receptor α; GPER, G protein-coupled estrogen receptor; , HAT, histone acetyltransferase; HIF-1α, hypoxia-inducible factor-1α; HDAC, histone deacetylase; HDACIs, HDAC inhibitors; HOXA10, homeobox A10; SF-1, steroidogenic factor-1.

expression. Since complex cellular responses to different HDACIs have been reported, their widely varying potency should be investigated. At present, the long-term safety and side-effects of epigenetic drugs for treating endometriosis are largely unknown; these should be carefully evaluated.

7. CONCLUSIONS

Possible roles of aberrant histone modifications in the pathogenesis of endometriosis are summarized in Figure 1. The aberrations discussed in this review give

rise to an important question: Are they the cause or merely the consequence of endometriosis? Since most of the previous studies reporting epigenetic aberrations in endometriosis were carried out cross-sectionally, the question of how the epigenetic changes occur in endometriosis is currently unknown. Further research is necessary to fully answer this question. Whatever the mechanisms may be, epigenetics appears to be a common denominator for the hormonal and immunological aberrations observed in endometriosis, and many aspects of this disorder can be explained from an epigenetics perspective.

Global gene expression studies in both human and animal models have shown that endometriotic lesions have aberrant gene expression profiles compared with the normal endometrium (124–128). The mechanisms at play have not been sufficiently studied, but the epigenetic regulation of gene expression is emerging as a key player in endometriosis (129). Thus, the current knowledge of the pathophysiology of endometriosis supports the involvement of both genetic and epigenetic phenomena in inducing changes in gene expression that lead to disease. Further research with other candidate genes that are epigenetically modified in endometriosis may elucidate the disease pathogenesis.

In conclusion, recent findings on aberrant histone modifications in endometriosis support the hypothesis that endometriosis should be recognized, at least in part, as an epigenetic disease. Among the epigenetic agents for the treatment of this disease, HDACIs are the most promising and most extensively studied. Some of the HDACIs are already FDA-approved and used in clinical practice for other diseases (116–118). A pilot clinical study has also reported the effectiveness of VPA on endometriosis (123). Larger controlled clinical trials are required to confirm the clinical benefits of HDACIs for the treatment of endometriosis and for the development of less-toxic and more effective HDACIs.

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9. REFERENCES

1. Giudice, L.C. & L.C. Kao: Endometriosis. *Lancet*, 364, 1789–1799 (2004)

2. Nasu, K., M. Nishida, Y. Kawano, A. Tsuno, W. Abe, A. Yuge, N. Takai & H. Narahara: Aberrant expression of apoptosis-related molecules in endometriosis: a possible mechanism underlying the pathogenesis of endometriosis. *Reprod Sci*, 18, 206–218 (2011)

3. Shin, J.C., H.L. Ross, S. Elias, D.D. Nguyen, D. Mitchell-Leef, J.L. Simpson & F.Z. Bischoff: Detection of chromosomal aneuploidy in endometriosis by multicolor fluorescence *in situ* hybridization (FISH). *Hum Genet*, 100, 401–406 (1997)

4. Gogusev, J., J. Bouquet de Joliniere, L. Telvi, M. Doussau, S. du Manoir, A. Stojkoski & M. Levardon: Detection of DNA copy number changes in human endometriosis by comparative genomic hybridization. *Hum Genet*, 105, 444–451 (1999)

5. Goumenou, A.G., D.A. Arvanitis, I.M. Matalliotakis, E.E. Koumantakis & D.A. Spandidos: Microsatellite DNA assays reveal an allelic imbalance in p16(Ink4), GALT, p53, and APOA2 loci in patients with endometriosis. *Fertil Steril*, 75, 160–165 (2001)

6. Bischoff, F.Z., M. Heard & J.L. Simpson: Somatic DNA alterations in endometriosis: high frequency of chromosome 17 and p53 loss in late-stage endometriosis. *J Reprod Immunol*, 55, 49–64 (2002)

7. Reik, W., W. Dean & J. Walter: Epigenetic reprogramming in mammalian development. *Science*, 293, 1089–1093 (2001)

8. Goldberg, A.D. & C.D. Allis: Bernstein E. Epigenetics: a landscape takes shape. *Cell*, 128, 635–638 (2007)

9. Jenuwein, T. & C.D. Allis: Translating the histone code. *Science*, 293, 1074–1080 (2001)

10. Jones, P.A. & S.B. Baylin: The fundamental role of epigenetic events in cancer. *Nat Rev Genet*, 3, 415–428 (2002)

11. Bartel, D.P: MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116, 281–297 (2004)

12. Pazin, M.J. & J.T. Kadonaga: What's up and down with histone deacetylation and transcription? *Cell*, 89, 325–328 (1997)

13. Takai, N. & H. Narahara: Human endometrial and ovarian cancer cells: histone deacetylase inhibitors exhibit antiproliferative activity, potently induce cell cycle arrest, and stimulate apoptosis. *Curr Med Chem*, 14, 2548–2553 (2007)

14. Kawano, Y., K. Nasu, H. Li, A. Tsuno, W. Abe, N. Takai & H. Narahara: Application of the histone deacetylase inhibitors for the treatment of endometriosis: histone modifications as pathogenesis and novel therapeutic target. *Hum Reprod*, 26, 2486–2498 (2011)

15. Nasu, K., Y. Kawano, Y. Tsukamoto, M. Takano, N. Takai, H. Li, Y. Furukawa, W. Abe, M. Moriyama & H. Narahara: Aberrant DNA methylation status of endometriosis: epigenetics as the pathogenesis, biomarker and therapeutic target. *J Obstet Gynaecol Res*, 37, 683–695 (2011)

16. Abe, W., K. Nasu, C. Nakada, Y. Kawano, M. Moriyama & H. Narahara: miR-196b targets c-myc and Bcl-2 expression, inhibits proliferation and induces apoptosis in endometriotic stromal cells. *Hum Reprod*, 28, 750–761 (2013)

17. Kawano, Y., K. Nasu, N. Hijiya, Y. Tsukamoto, K. Amada, W. Abe, K. Kai, M. Moriyama & H. Narahara: CCAAT/enhancer-binding protein α is epigenetically silenced by histone deacetylation in endometriosis and promotes the pathogenesis of endometriosis. *J Clin Endocrinol Metab*, 98, E1474-E1482 (2013)

18. Wu, Y. & S.W. Guo: Suppression of IL-1β-induced COX-2 expression by trichostatin A (TSA) in human endometrial stromal cells. *Eur J Obstet Gynecol Reprod Biol*, 135, 88–93 (2007)

19. Lu, Y., J. Nie, X. Liu, Y. Zheng & S.W. Guo: Trichostatin A, a histone deacetylase inhibitor, reduces lesion growth and hyperalgesia in experimentally induced endometriosis in mice. *Hum Reprod*, 25, 1014–1025 (2010)

20. Wu, Y., A. Starzinski-Powitz & S.-W. Guo: Constitutive and Tumor Necrosis Factor-Alpha-Stimulated Activation of Nuclear Factor-KappaB in Immortalized Endometriotic Cells and Their Suppression by Trichostatin A. *Gynecol Obstet Invest*, 70, 23–33 (2010)

21. Monteiro, J.B., M. Colón-Díaz, M. García, S. Gutierrez, M. Colón, E. Seto, J. Laboy & I. Flores: Endometriosis is characterized by a distinct pattern of histone 3 and histone 4 lysine modifications. *Reprod Sci*, (Epub ahead of print) (2013)

22. Xiaomeng, X., Z. Ming, M. Jiezhi & F. Xiaoling: Aberrant histone acetylation and methylation levels in woman with endometriosis. *Arch Gynecol Obstet*, 287, 487–494 (2013)

23. Cress, W.D. & E. Seto: Histone deacetylases, transcriptional control, and cancer. *J Cell Physiol*, 184, 1–16 (2000)

24. Bertrand, P: Inside HDAC with HDAC inhibitors. *Eur J Med Chem*, 45, 2095–2116 (2010)

25. Munro, S.K., C.M. Farquhar, M.D. Mitchell & A.P. Ponnampalam: Epigenetic regulation of endometrium during the menstrual cycle. *Mol Hum Reprod*, 16, 297–310 (2010)

26. De Ruijter, A.J., A.H. van Gennip, H.N. Caron, S. Kemp & A.B. van Kuilenburg: Histone deacetylases (HDACs): characterization of the classical HDAC family. *Biochem J*, 370, 737–749 (2003)

27. Peinado, H., E. Ballestar, M. Esteller & A. Cano: Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. *Mol Cell Biol*, 24, 306–319 (2004)

28. Grzenda, A., G. Lomberk, J.S. Zhang & R. Urrutia: Sin3: master scaffold and transcriptional corepressor. *Biochim Biophys Acta*, 1789, 443–450 (2009)

29. Hayashi, A., A. Horiuchi, N. Kikuchi, T. Hayashi, C. Fuseya, A. Suzuki, I. Konishi & T. Shiozawa: Type-specific roles of histone deacetylase (HDAC) overexpression in ovarian carcinoma: HDAC1 enhances cell proliferation and HDAC3 stimulates cell migration with downregulation of E-cadherin. *Int J Cancer*, 127, 1332–1346 (2010)

30. Wilting, R.H., E. Yanover, M.R. Heideman, H. Jacobs, J. Horner, J. van der Torre, R.A. DePinho & J.H. Dannenberg: Overlapping functions of Hdac1 and Hdac2 in cell cycle regulation and haematopoiesis. *EMBO J*, 29, 2586–2597 (2010)

31. Yamaguchi, T., F. Cubizolles, Y. Zhang, N. Reichert, H. Kohler, C. Seiser & P. Matthias: Histone deacetylases 1 and 2 act in concert to promote the G1-to-S progression. *Genes Dev*, 24, 455–469 (2010)

32. Krusche, C.A., A.J. Vloet, I. Classen-Linke, U. von Rango, H.M. Beier & J. Alfer: Class I histone deacetylase expression in the human cyclic endometrium and endometrial adenocarcinomas. *Hum Reprod*, 22, 2956–2966 (2007)

33. Colón-Díaz, M., P. Báez-Vega, M. García, A Ruiz, J.B. Monteiro, J. Fourquet, M. Bayona, C. Alvarez-Garriga, A. Achille, E. Seto, & I. Flores: HDAC1 and HDAC2 are differentially expressed in endometriosis. *Reprod Sci*, 19, 483–492 (2012)

34. Samartzis, E.P., A. Noske, N. Samartzis, D. Fink & P. Imesch: The Expression of Histone Deacetylase 1, But Not Other Class I Histone Deacetylases, Is Significantly Increased in Endometriosis. *Reprod Sci*, (Epub ahead of print) (2013)

35. Brandenberger, A.W., D.I. Lebovic, M.K. Tee, I.P. Ryan., J.F. Tseng, R.B. Jaffe & R.N. Taylor: Oestrogen receptor (ER)-alpha and ER-beta isoforms in normal endometrial and endometriosis-derived stromal cells. *Mol Hum Reprod*, 5, 651–655 (1999)

36. Fujimoto, J., R. Hirose, H. Sakaguchi & T. Tamaya: Expression of oestrogen receptor-alpha and -beta in ovarian endometriomata. *Mol Hum Reprod*, 5, 742–747 (1999)

37. Xue, Q., Z. Lin, Y.H. Cheng, C.C. Huang, E. Marsh, P. Yin, M.P. Milad, E. Confino, S. Reierstad, J. Innes & S.E. Bulun: Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. *Biol Reprod*, 77, 681–687 (2007)

38. Kuiper, G.G., B. Carlsson, K. Grandien, E. Enmark, J. Häggblad, S. Nilsson & J.A. Gustafsson: Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology*, 138, 863–870 (1997)

39. Tong, W., R. Perkins, L. Xing, W.J. Welsh & D.M. Sheehan: QSAR models for binding of estrogenic compounds to estrogen receptor alpha and beta subtypes. *Endocrinology*, 138, 4022–4025 (1997)

40. Prossnitz, E.R., & M. Maggiolini: Mechanisms of estrogen signaling and gene expression via GPR30. *Mol Cell Endocrinol*, 308, 32–38 (2009)

41. Maggiolini, M. & D. Picard: The unfolding stories of GPR30, a new membranebound estrogen receptor. *J Endocrinol*, 204, 105–114 (2010)

42. Samartzis, N., E.P. Samartzis, A. Noske, A. Fedier, K.J. Dedes, R. Caduff, D. Fink & P. Imesch: Expression of the G protein-coupled estrogen receptor (GPER) in endometriosis: a tissue microarray study. *Reprod Biol Endocrinol*, 10: 30 (2012)

43. Heublein, S., M. Lenhard, T. Vrekoussis, J. Schoepfer, C. Kuhn, K. Friese, D. Mayr, A. Makrigiannakis & U. Jeschke: The G-protein coupled estrogen receptor (GPER) is expressed in normal human ovaries and is upregulated in ovarian endometriosis and pelvic inflammatory disease involving the ovary. *Reprod Sci*, 19, 1197–1204 (2012)

44. Imesch P., E.P. Samartzis, K.J. Dedes, D. Fink & A. Fedier: Histone deacetylase inhibitors down-regulate G-protein-coupled estrogen receptor and the GPER-antagonist G-15 inhibits proliferation in endometriotic cells. *Fertil Steril*, 100, 770-776 (2013)

45. Rice, D.A., A.R. Mouw, A.M. Bogerd & K.L. Parker: A shared promoter element regulates the expression of three steroidogenic enzymes. *Mol Endocrinol*, 5, 1552–1561 (1991)

46. Morohashi, K., S. Honda, Y. Inomata, H. Handa & T. Omura: A common trans-acting factor, Ad4-binding protein, to the promoters of steroidogenic P-450s. J Biol Chem, 267, 17913–17919 (1992)

47. Zeitoun, K., K. Takayama, M.D. Michael & S.E. Bulun: Stimulation of aromatase P450 promoter (II) activity in endometriosis and its inhibition in endometrium are regulated by competitive binding of steroidogenic factor-1 and chicken ovalbumin upstream promoter transcription factor to the same cis-acting element. Mol Endocrinol, 13, 239–253 (1999)

48. Xue, Q., Z. Lin, P. Yin, M.P. Milad, Y.-H. Cheng, E. Confino, S. Reierstad & S.E. Bulun: Transcriptional Activation of steroidogenic factor-1 by hypomethylation of the 5' CpG island in endometriosis. J Clin Endocrinol Metab, 92, 3261–3232 (2007)

49. Gehring W.J: Homeo boxes in the study of development. *Science*, 236, 1245–1252 (1987)

50. Taylor, H.S., A. Arici, D. Olive & P. Igarashi: HOXA10 is expressed in response to sex steroids at the time of implantation in the human endometrium. *J Clin Invest*, 101, 1379–1384 (1998)

51. Taylor, H.S., C. Bagot, A. Kardana, D. Olive & A. Arici: HOX gene expression is altered in the endometrium of women with endometriosis. *Hum Reprod*, 14, 1328–1331 (1999)

52. Gui, Y., J. Zhang, L. Yuan & B.A. Lessey: Regulation of HOXA-10 is and its expression in normal and abnormal endometrium. *Mol Hum Reprod*, 5, 866–873 (1999)

53. Wu, Y., G. Halverson, Z. Basir, E. Strawn, P. Yan & S.W. Guo: Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. *Am J Obstet Gynecol*, 193, 371–380 (2005)

54. Ramji, D.P. & P. Foka: CCAAT/enhancer-binding proteins: structure, function and regulation. *Biochem J*, 365, 561–575 (2002)

55. Yang, S., Z. Fang, T. Suzuki, H. Sasano, J. Zhou, B. Gurates, M. Tamura, K. Ferrer & S. Bulun: Regulation of aromatase P450 expression in endometriotic and endometrial stromal cells by CCAAT/enhancer binding proteins (C/EBPs): decreased C/EBPbeta in endometriosis is associated with overexpression of aromatase. *J Clin Endocrinol Metab*, 87, 2336–2345 (2002)

56. Wu, M.H., K.F. Chen, S.C. Lin, C.W. Lgu & S.J. Tsai: Aberrant expression of leptin in human endometriotic stromal cells is induced by elevated levels of hypoxia inducible factor-1alpha. *Am J Pathol*, 170, 590–598 (2007)

57. Kim, S.H., J.W. Jeong, J.A. Park, J.W. Lee, J.H. Seo, B.K. Jung, M.K. Bae & K.W. Kim: Regulation of the HIF-1α stability by histone deacetylases. *Oncol Rep*, 17, 647–651 (2007)

58. Ellis, L., H. Hammers & R. Pili: Targeting tumor angiogenesis with histone deacetylase inhibitors. *Cancer Lett*, 280, 145–153 (2009)

59. Imesch, P., D. Fink & A. Fedier: Romidepsin reduces histone deacetylase activity, induces acetylation of histones, inhibits proliferation, and activates apoptosis in immortalized epithelial endometriotic cells. *Fertil Steril*, 94, 2838–2842 (2010)

60. Imesch, P., E.P. Samartzis, M. Schneider, D. Fink & A. Fedier: Inhibition of transcription, expression, and secretion of the vascular epithelial growth factor in human epithelial endometriotic cells by romidepsin. *Fertil Steril*, 95, 1579–1583 (2011)

61. Serrano, M., G.J. Hannon & D. Beach: A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature*, 366, 704–707 (1993)

62. Lowe, S.W. & C.J. Sherr: Tumor suppression by Ink4a-Arf: progress and puzzles. *Curr Opin Genet Dev*, 13, 77–83 (2003)

63. Brugarolas, J., C. Chandrasekaran, J.I. Gordon, D. Beach, T. Jacks & G.J. Hannon: Radiation-induced cell cycle arrest compromised by p21 deficiency. *Nature*, 377, 552–557 (1995)

64. Deng, C., P. Zhang, J.W. Harper, S.J. Elledge & P. Leder: Mice lacking $p21^{CIP1/WAF1}$ undergo normal development, but are defective in G1 checkpoint control. *Cell*, 82, 675–684 (1995)

65. Takai, N., M. Kawamata, D. Gui, J.W. Said, I. Miyakawa & H.P. Koeffler: Human ovarian carcinoma

cells: histone deacetylase inhibitors exhibit antiproliferative activity and potently induce apoptosis. *Cancer*, 101, 2760–2770 (2004)

66. Chen, J., P.K. Jackson, M.W. Kirschner & A. Dutta: Separate domains of p21 involved in the inhibition of Cdk kinase and PCNA. *Nature*, 374, 386–388 (1995)

67. Luo, Y., J. Hurwitz & J. Massague: Cell-cycle inhibition by independent CDK and PCNA binding domains in $p21^{Cip1}$. *Nature*, 375, 159–161 (1995)

68. Moldovan, G.L., B. Pfander & S. Jentsch: PCNA, the maestro of the replication fork. *Cell*, 129, 665–679 (2007)

69. Mandal, M., D. Bandyopadhyay, T.M. Goepfert & R. Kumar: Interferon-induces expression of cyclin-dependent kinase-inhibitors $p21^{WAF1}$ and $p27^{Kipl}$ that prevent activation of cyclin-dependent kinase by CDK-activating kinase (CAK). *Oncogene*, 16, 217–225 (1998)

70. Smits, V.A., R. Klompmaker, T. Vallenius, G. Rijksen, T.P. Mäkela & R.H. Medema: p21 inhibits Thr161 phosphorylation of Cdc2 to enforce the G2 DNA damage checkpoint. *J Biol Chem*, 275, 30638–30643 (2000)

71. Abbas, T., S. Jha, N.E. Sherman & A. Dutta: Autocatalytic phosphorylation of CDK2 at the activating Thr160. *Cell Cycle*, 6, 843–852 (2007)

72. Katayose, Y., M. Kim, A.N. Rakkar, Z. Li, K.H. Cowan & P. Seth: Promoting apoptosis: a novel activity associated with the cyclin-dependent kinase inhibitor p27. *Cancer Res*, 57, 5441–5445 (1997)

73. Keyomarsi, K., S.L. Tucker, T.A. Buchholz, M. Callister, Y. Ding, G.N. Hortobagyi, I. Bedrosian, C. Knickerbocker, W. Toyofuku, M. Lowe, T.W. Herliczek & S.S. Bacus: Cyclin E and survival in patients with breast cancer. *N Engl J Med*, 347, 1566–1575 (2002)

74. Bloom, J. & M. Pagano: Deregulated degradation of the CDK inhibitor p27 and malignant transformation. *Semin Cancer Biol*, 13, 41–47 (2003)

75. Chu, I.M., L. Hengst & J.M. Slingerland: The Cdk inhibitor p27 in human cancer: prognostic potential and relevance to anticancer therapy. *Nat Rev Cancer*, 8, 253–267 (2008)

76. Levkau, B., H. Koyama, E.W. Raines, B.E. Clurman, B. Herren, K. Orth, J.M. Roberts & R. Ross: Cleavage of p21Cip1/Waf1 and p27Kip1mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade. *Mol Cell*, 1, 553–563 (1998)

77. Besson, A., S.F. Dowdy & J.M. Roberts: CDK inhibitors: cell cycle regulators and beyond. *Dev Cell*, 14, 159–169 (2008)

78. St Croix, B., V.A. Florenes, J.W. Rak, M. Flanagan, N. Bhattacharya, J.M. Slingerland & R.S. Kerbel: Impact of the cyclin-dependent kinase inhibitor p27Kip1 on resistance of tumor cells to anticancer agents. *Nat Med*, 2, 1204–1210 (1996)

79. Chaturvedi, P., W.K. Eng, Y. Zhu, M.R. Mattern, R. Mishra, M.R. Hurle, X. Zhang, R.S. Annan, Q. Lu, L.F. Faucette, G.F. Scott, X. Li, S.A. Carr, R.K. Johnson, J.D. Winkler & B.B. Zhou: Mammalian Chk2 is a downstream effector of the ATM-dependent DNA damage checkpoint pathway. *Oncogene*, 18, 4047–4054 (1999)

80. Bartek, J. & J. Lukas: Mammalian G1- and S- phase checkpoints in response to DNA damage. *Curr Opin Cell Biol*, 13, 738–747 (2001)

81. Kato, N., H. Fujimoto, A. Yoda, I. Oishi, N. Matsumura, T. Kondo, J. Tsukada, Y. Tanaka, M. Imamura & Y. Minami: Regulation of Chk2 gene expression in lymphoid malignancies: involvement of epigenetic mechanisms in Hodgkin's lymphoma cell lines. *Cell Death Differ*, 11, S153–S161 (2004)

82. Chehab, N.H., A. Malikzay, M. Appel & T.D. Halazonetis: Chk2/hCds1 functions as a DNA damage checkpoint in G1 by stabilizing p53. *Genes Dev*, 14, 278–288 (2000)

83. Bartek, J. & J. Lukas: Chk1 and Chk2 kinases in checkpoint control and cancer. *Cancer Cell*, 3, 421–429 (2003)

84. Turnbull, C., S. Seal, A. Renwick, M. Warren-Perry, D. Hughes, A. Elliott, D. Pernet, S. Peock, J.W. Adlard, J. Barwell, J. Berg, A.F. Brady, C. Brewer, G. Brice, C. Chapman, J. Cook, R. Davidson, A. Donaldson, F. Douglas, L. Greenhalgh, A. Henderson, L. Izatt, A. Kumar, F. Lalloo, Z. Miedzybrodzka, P.J. Morrison, J. Paterson, M. Porteous, M.T. Rogers, S. Shanley, L. Walker; Breast Cancer Susceptibility Collaboration (UK), EMBRACE, M. Ahmed, D. Eccles, D.G. Evans, P. Donnelly, D.F. Easton, M.R. Stratton & N. Rahman: Gene-gene interactions in breast cancer susceptibility. *Hum Mol Genet*, 21, 958–962 (2012)

85. Fuchs, P., S. Strehl, M. Dworzak, A. Himmler & P.F. Ambros: Structure of the human TNF receptor 1 (p60) gene (TNFR1) and localization to chromosome 12p13. *Genomics*, 13, 219–224 (1992)

86. Behrmann, I., H. Walczak & P.H. Krammer: Structure of the human APO-1 gene. *Eur J Immunol*, 24, 3057–3062 (1994)

87. Chinnaiyan, A.M., K. O'Rourke, G.L. Yu, R.H. Lyons, M. Garg, D.R. Duan, L. Xing, R. Gentz, J. Ni & V.M. Dixit: Signal transduction by DR3, a death domain-containing receptor related to TNFR-1 and CD95. *Science*, 274, 990–992 (1996)

88. Pan, G., K. O'Rourke, A.M. Chinnaiyan, R. Gentz, R. Ebner, J. Ni & V.M. Dixit: The receptor for the cytotoxic ligand TRAIL. *Science*, 276, 111–113 (1997)

89. Pan, G., J. Ni, Y.F. Wei, G. Yu, R. Gentz & V.M. Dixit: An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science*, 277, 815–818 (1997)

90. Pan, G., J.H. Bauer, V. Haridas, S. Wang, D. Liu, G. Yu, C. Vincenz, B.B. Aggarwal, J. Ni & V.M. Dixit: Identification and functional characterization of DR6, a novel death domain-containing TNF receptor. *FEBS Lett*, 431, 351–356 (1998)

91. Smith, C.A., T. Farrah & R.G. Goodwin: The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. *Cell*, 76, 959–962 (1994)

92. Jeremias, I. & K.M. Debatin: TRAIL induces apoptosis and activation of NFkappaB. *Eur Cytokine Netw*, 9, 687–8 (1998)

93. Schulze-Osthoff K., D. Ferrari, M. Los, S. Wesselborg & E. Peter M: Apoptosis signaling by death receptors. *Eur J Biochem*, 254, 439–459 (1998)

94. Aggarwal, B.B: Signalling pathways of the TNF superfamily: a double-edged sword. *Nature Rev Immunol*, 3, 745–756 (2003)

95. Elrod, H.A. & S.Y. Sun: Modulation of death receptors by cancer therapeutic agents. *Cancer Biol Ther*, 7, 163–173 (2008)

96. Locksley, R.M., N. Killeen & M.J. Lenardo: The TNF and TNF receptor superfamilies: integrating mammalian biology. *Cell*, 104, 487–501 (2001)

97. Bossen C., K. Ingold, A. Tardivel, J.L. Bodmer, O. Gaide, S. Hertig, C. Ambrose, J. Tschopp & P. Schneider: Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. *J Biol Chem*, 281, 13964–13971 (2006)

98. Wilson, N.S., V.M. Dixit & A. Ashkenazi: Death receptor signal transducers: nodes of coordination in immune signaling networks. *Nat Immunol*, 10, 348–355 (2009)

99. Liu, J., S. Na, A. Glasebrook, N. Fox, P.J. Solenberg, Q. Zhang, H.Y. Song & D.Yang: Enhanced CD4+ T cell proliferation and Th2 cytokine production in DR6-deficient mice. *Immunity*, 15, 23–34 (2001)

100. Zhao, H., M. Yan, H. Wang, S. Erickson, I.S. Grewal, V.M. Dixit: Impaired c-Jun amino terminal kinase activity and T cell differentiation in death receptor 6-deficient mice. *J Exp Med*, 194, 1441–1448 (2001)

101. Schmidt, C. S., J. Liu, T. Zhang, H.Y. Song, G. Sandusky, K. Mintze, R.J. Benschop, A. Glasebrook, D.D. Yang & S. Na: Enhanced B cell expansion, survival, and humoral responses by targeting death receptor 6. *J Exp Med*, 197, 51–62 (2003)

102. Nikolaev, A., T. McLaughlin, D.D. O'Leary, M. Tessier-Lavigne: APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature*, 457, 981–989 (2009)

103. Mi, S., X.H. Lee, Y.H. Hu, B.X. Ji, Z.H. Shao, W.X. Yang, G. Huang, L. Walus, K. Rhodes, B. J. Gong, R.H. Miller & R.B. Pepinsky: Death receptor 6 negatively regulates oligodendrocyte survival, maturation and myelination. *Nat Med*, 17, 816–821 (2011)

104. Kai, K., K. Nasu, Y. Kawano, Y. Aoyagi, Y. Tsukamoto, N. Hijiya, W. Abe, M. Okamoto, M. Moriyama & H. Narahara: Death Receptor 6 is Epigenetically Silenced by Histone Deacetylation in Endometriosis and Promotes the Pathogenesis of Endometriosis. *Am J Reprod Immunol*, 70, 485–496 (2013)

105. Gumbiner, B.M: Regulation of cadherin adhesive activity. *J Cell Biol*, 148, 399–404 (2000)

106. Frixen, U.H., J. Behrens, M. Sachs, G. Eberle, B. Voss, A. Warda, D. Löchner & W. Birchmeier: E-cadherin-mediated cell-cell adhesion prevents invasiveness of human carcinoma cells. *J Cell Biol*, 113, 173–185 (1991)

107. Starzinski-Powitz, A., R. Gaetje, A. Zeitvogel, S. Kotzian, H. Handrow-Metzmacher, G. Herrmann, E. Fanning & R. Baumann: Tracing cellular and molecular mechanisms involved in endometriosis. *Hum Reprod Update*, 4, 724–729 (1998)

108. Wu, Y., A. Starzinski-Powitz & S.-W. Guo: Trichostatin A, a histone deacetylase inhibitor, attenuates invasiveness and reactivates E-cadherin expression in immortalized endometriotic cells. *Reprod Sci*, 14, 374–382 (2007)

109. Wu, Y., A. Starzinski-Powitz & S.W. Guo: Capsaicin inhibits proliferation of endometriotic cells *in vitro*. *Gynecol Obstet Invest*, 66, 59–62 (2008)

110. Practice Committee of the American Society for Reproductive Medicine: Endometriosis and infertility. *Fertil Steril*, 81, 1441–1446 (2004)

111. Nasu, K., A. Tsuno, A. Yuge, Y. Kawano & H. Narahara: Combined oral contraceptives for the medical treatment of endometriosis-associated pain. *Recent Adv Endocrinol Metab*, 1, 1–14 (2009)

112. Lessey, B.A: Medical management of endometriosis and infertility. *Fertil Steril*, 73, 1089–1096 (2000)

113. Bulun, S.E., Z. Lin, G. Imir, S. Amin, M. Demura, B. Yilmaz, R. Martin, H. Utsunomiya, S. Thung, B. Gurates, M. Tamura, D. Langoi & S. Deb: Regulation of aromatase expression in estrogen-responsive breast and uterine

disease: from bench to treatment. *Pharmacol Rev*, 57, 359–383 (2005)

114. Bergqvist, A: A comparative study of the acceptability and effect of goserelin and nafarelin on endometriosis. *Gynecol Endocrinol*, 14, 425–432 (2000)

115. Yoo, C.B. & P.A. Jones: Epigenetic therapy of cancer: past, present and future. *Nat Rev Drug Discov*, 5, 37–50 (2006)

116. Duenas-Gonzalez, A., M. Candelaria, C. Perez-Plascencia, E. Perez-Cardenas, E. de la Cruz-Hernandez & L.A. Herrera: Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. *Cancer Treat Rev*, 34, 206–222 (2008)

117. Mann, B.S., J.R. Johnson, K. He, R. Sridhara, S. Abraham, B.P. Booth, L. Verbois, D.E. Morse, J.M. Jee, S. Pope, R.S. Harapanhalli, R. Dagher, A. Farrell, R. Justice & R. Pazdur: Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. *Clin Cancer Res*, 13, 2318–2322 (2007)

118. Gerstner, T., N. Bell & S. Konig: Oral valproic acid for epilepsy—long-term experience in therapy and side effects. *Exp Opin Pharmacother*, 9, 285–292 (2008)

119. Wu, Y & S.W. Guo: Histone deacetylase inhibitors trichostatin A and valproic acid induce cell cycle arrest and p21 expression in immortalized human endometrial stromal cells. *Eur J Obstet Gynecol Reprod Biol*, 137, 198–203 (2008)

120. Wu, Y., A. Starzinski-Powitz & S.-W. Guo: Prolonged stimulation with tumor necrosis factor-α induced partial methylation at PR-B promoter in immortalized epithelial-like endometriotic cells. *Fertil Steril*, 90, 234–237 (2008)

121. Wu, Y & S.W. Guo: Peroxisome proliferator-activated receptor-gamma and retinoid X receptor agonists synergistically suppress proliferation of immortalized endometrial stromal cells. *Fertil Steril*, 91 Suppl, 2142–2147 (2009)

122. Liu, M., X. Liu, Y. Zhang & S.W. Guo: Valproic acid and progestin inhibit lesion growth and reduce hyperalgesia in experimentally induced endometriosis in rats. *Reprod Sci*, 19, 360–373 (2012)

123. Liu, X & S.W. Guo: A pilot study on the off-label use of valproic acid to treat adenomyosis. *Fertil Steril*, 89, 246–250 (2008)

124. Eyster, K.M., A.L. Boles, J.D. Brannian & K.A. Hansen: DNA microarray analysis of gene expression markers of endometriosis. *Fertil Steril*, 77, 38–42 (2002)

125. Kao, L.C., A. Germeyer, S. Tulac, S. Lobo, J.P. Yang, R.N. Taylor, K. Osteen, B.A. Lessey & L.C. Giudice: Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. *Endocrinology*, 144, 2870–2881 (2003)

126. Matsuzaki, S., M. Canis, J.L. Pouly, R. Botchorishvili, P.J. Dechelotte & G. Mage: Differential expression of genes in eutopic and ectopic endometrium from patients with ovarian endometriosis. *Fertil Steril*, 86, 548–553 (2006)

127. Flores, I., E. Rivera, L.A. Ruiz, O.I. Santiago, M.W. Vernon & C.B. Appleyard: Molecular profiling of experimental endometriosis identified gene expression patterns in common with human disease. *Fertil Steril*, 87, 1180–1199 (2007)

128. Konno, R., H. Fujiwara, S. Netsu, K. Odagiri, M. Shimane, H. Nomura & M. Suzuki: Gene expression profiling of the rat endometriosis model. *Am J Reprod Immunol*, 58, 330–343 (2007)

129. Guo, S.W: Epigenetics of endometriosis. *Mol Hum Reprod*, 15, 587–607 (2009)

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