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Review

Histone deacetylases and cancer

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ABSTRACT

Reversible acetylation of histone and non-histone proteins is one of the most abundant post-translational modifications in eukaryotic cells. Protein acetylation and deacetylation are achieved by the antagonistic actions of two families of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs). Aberrant protein acetylation, particularly on histones, has been related to cancer while abnormal expression of HDACs has been found in a broad range of cancer types. Therefore, HDACs have emerged as promising targets in cancer therapeutics, and the development of HDAC inhibitors (HDIs), a rapidly evolving area of clinical research. However, the contributions of specific HDACs to a given cancer type remain incompletely understood. The aim of this review is to summarize the current knowledge concerning the role of HDACs in cancer with special emphasis on what we have learned from the analysis of patient samples.

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1. Protein acetylation: a brief introduction

Chromatin, the higher-order structure of DNA and protein, forms a barrier for gene transcription. The basic unit of chromatin is the nucleosome, which comprises 147 bp of DNA wrapped around a histone core containing two copies each of histones H2A, H2B, H3 and H4. This core is important for establishing interactions between nucleosomes and within the nucleosome itself (Khorasanizadeh, 2004). Chromatin can adopt different structural conformations depending on the epigenetic modifications that occur in the DNA and in the histone tails protruding from the nucleosomes. Changes in the chromatin status of specific genes can lead to their repression or activation. Several histone post-translational modifications have been described, including acetylation, methylation, phosphorylation and sumoylation (Bannister

and Kouzarides, 2011). Histone post-translational modifications constitute the so-called “histone code” that is read and recognized by additional proteins in order to regulate gene expression (Strahl and Allis, 2000).

Around fifty years ago, Vincent Allfrey and colleagues discovered the lysine acetylation of histones, demonstrating that acetylation of the ϵ -amino group of lysine residues on histones could play a role in gene expression (Allfrey et al., 1964; Gershey et al., 1968). The acetylation neutralizes the positive charge of the histone lysine residues, relaxing the chromatin conformation and enabling greater accessibility of the transcription machinery (Haberland et al., 2009). Protein acetylation is therefore generally associated with gene activation. In contrast, the removal of acetyl groups from histones induces chromatin condensation and gene transcriptional repression (Haberland et al., 2009). It is well established that

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lysine acetylation also occurs in a considerable number of non-histone proteins, such as transcription factors and cytoplasmic proteins, and affects gene transcription and other cellular processes (Peng and Seto, 2011). Lysine acetylation is a reversible modification controlled by the antagonistic actions of two types of enzymes, histone acetylases (HATs) and histone deacetylases (HDACs) (Bannister and Kouzarides, 2011). HATs catalyze the transfer of acetyl groups from acetyl CoA to the ϵ -amino group of the lysine residue. Conversely, HDACs promote the removal of the acetyl group from the acetylated residue, releasing an acetate molecule (Figure 1) (for recent reviews on HDACs targets see (Bosch-Presegue and Vaquero, 2011; Peng and Seto, 2011; Reichert et al., 2012)).

2. Histone deacetylases: an overview

HDACs have emerged as crucial transcriptional co-repressors in highly diverse physiological and pathological systems. To date, 18 human HDACs have been identified and grouped into four classes on the basis of their homology with yeast proteins. Class I HDACs (HDAC1, 2, 3, and 8) share high homology with the yeast transcriptional regulator RPD3, class II HDACs are closely related to HDA1 (HDAC4, 5, 6, 7, 9, and 10), class III HDACs, also called sirtuins, are homologous with Sir2 (SIRT1, 2, 3, 4, 5, 6, and 7), and class IV HDAC (HDAC11) is homologous with class I and II enzymes. Class II HDACs are further subdivided into class IIa (HDAC4, 5, 7, 9) and class IIb (HDAC6 and 10) forms (Haberland et al., 2009; Parra and Verdin, 2010) (Figure 2). Class I, II and VI HDACs are also referred to as “classical” HDACs and are Zn^{2+} -dependent enzymes whereas sirtuins require NAD^+ as a cofactor.

Class I HDACs are ubiquitously expressed in all tissues. They are predominantly localized in the nuclear compartment of the cell and exert a strong catalytic effect on histone lysine residues. HDAC1 and HDAC2 are highly similar and are involved in many cellular processes such as proliferation, cell cycle and apoptosis (Segre and Chiocca, 2011). In the whole organism they seem to have a critical role in development and physiology (Reichert et al., 2012). HDAC3 plays a role in cell cycle processes and DNA damage response (Reichert et al., 2012). Finally, HDAC8 is predominantly found in the cytosol and is expressed in cells showing smooth muscle differentiation

(Waltregny et al., 2005). The protein structure of Class I HDACs is characterized by a highly conserved deacetylase domain flanked by short amino- and carboxy-terminal extensions (Yang and Seto, 2008) (Figure 2). Class I HDACs are found as part of multi-protein complexes recruited to target genes to mediate repression. HDAC1 and HDAC2 are catalytic subunits of the Sin3, Mi-2/NurD and CoREST complexes, whereas HDAC3 is mainly recruited by the N-CoR/SMRT complex. HDAC8 has not so far been described as being a member of any protein complex (Yang and Seto, 2008).

Unlike class I HDACs, class IIa HDACs are expressed in a tissue-specific manner and are involved in differentiation and development. They exert their transcriptional repressive function in skeletal, cardiac, and smooth muscle, bone, the immune system, the vascular system, and the brain among others. A peculiarity of class IIa HDACs is that, together with the conserved deacetylase domain, they possess a long regulatory N-terminal domain that mediates their interactions with tissue-specific transcription factors and co-repressors (Parra and Verdin, 2010; Yang and Seto, 2008) (Figure 2). The amino-terminal domain contains highly conserved serine residues that are subjected to phosphorylation. Signal-dependent phosphorylation of class IIa HDACs is a critical event that determines whether they are localized in the nucleus or cytoplasm and, therefore, their ability to act as transcriptional co-repressors in the nuclear compartment (Parra and Verdin, 2010; Yang and Seto, 2008). Although class IIa HDACs have a highly conserved histone deacetylase domain, their specific catalytic activity remains elusive. This is a paradigm in the field that awaits exploration. Class IIa HDACs have been found as part of the repressor complex SMRT/N-CoR (Fischle et al., 2002; Huang et al., 2000). HDAC6 and HDAC10 are the two members of the class IIb subfamily. HDAC6 is mainly found in the cytoplasm, where its main target is α -tubulin, and contains two deacetylase domains and a carboxy-terminus zinc finger (Hubbert et al., 2002; Yang and Seto, 2008). HDAC10 is found in the nucleus and cytoplasm and also contains a second deacetylase domain (Hubbert et al., 2002; Yang and Seto, 2008). Its specific substrates remain unknown.

Sirtuins are widely expressed and have a broad range of biological functions, such as the regulation of oxidative stress, DNA repair, regulation of metabolism and aging, among others (Bosch-Presegue and Vaquero, 2011; Saunders and

Figure 1 – Representative scheme of lysine deacetylation by HDACs. In the presence of a water molecule, HDACs catalyze the removal of the acetyl group from lysine regenerating the ϵ -amino group and releasing an acetate molecule.

Figure 2 – Human HDACs superfamily. HDACs are grouped into four different classes according to sequence similarity and homology to yeast proteins. Specific domains present in different members of each HDAC subfamily are represented in the illustrating cartoon.

Verdin, 2007). Sirtuins are localized in different cellular compartments: SIRT1, SIRT6 and SIRT7 are localized in the nucleus, SIRT2 is found in the cytosol, and SIRT3, 4 and 5 are mainly found in the mitochondria (Bosch-Presegue and Vaquero, 2011; Saunders and Verdin, 2007).

HDAC11 is currently the only member of the Class IV HDAC subfamily. HDAC11 contains conserved residues in the catalytic core regions that are shared by both class I and class II HDACs (Gao et al., 2002). Its expression is enriched in kidney, brain, testis, heart and skeletal muscle, but its function has been little studied. It has been associated with oligodendrocyte development and immune system response (Liu et al., 2009; Villagra et al., 2009).

3. Histone deacetylases and cancer

Mutation and/or aberrant expression of various HDACs have often been observed in human disease, in particular cancer, making them important therapeutic targets for many human cancers. Therefore, the global pattern of histone acetylation is deregulated in cancer. Indeed, Esteller and colleagues have reported that cancer cells undergo a loss of acetylation of histone H4 at lysine 16 indicating that HDAC activity is critical in establishing the tumor phenotype (Fraga et al., 2005). In

pathological situations where classical HDACs are overrepresented, histone deacetylase inhibitors (HDIs) have emerged as promising cancer therapeutic agents. To date, two HDIs have been approved for cancer therapy (cutaneous T-cell lymphoma); vorinostat (SAHA, Zolinza) and romidepsin (FK228, Istodax) by the Food and Drug Administration (FDA), and several others are undergoing clinical trials (Khan and La Thangue, 2012). However, most HDIs are disadvantaged by their lack of enzyme specificity and can cause a broad range of side effects. Moreover, it is noteworthy to mention that the contribution of HDACs to cancer can be due to mechanisms other than overexpression. In fact, HDACs may also present truncating or inactivating mutations. In addition, HDACs can be aberrantly recruited to target genes via their interaction with fusion proteins, as is the case in certain leukemias. In this scenario, alternative therapeutic agents will need to be explored. Below, we summarize our current knowledge of the contribution of HDACs to cancer from two perspectives; their expression in cancer patients and their mechanism of action in established cancer cell lines.

3.1. Class I HDACs

All the members of the Class I subfamily of HDACs are deregulated in many cancers. In several studies analyzing patient

cancer samples, overexpression of HDAC1 has been found in gastric, breast, pancreatic, hepatocellular, lung and prostate carcinomas and in most of the cases HDAC1 up-regulation associates with poor prognosis (Choi et al., 2001, 2010; Minamiya et al., 2011; Zhang et al., 2005). Other studies have reported high expression of HDAC1, HDAC2 and HDAC3 in renal cell cancer, colorectal and gastric cancer as well as in classical Hodgkin's lymphoma (Adams et al., 2010; Fritzsche et al., 2008; Weichert et al., 2008). In a different study analyzing breast tumors, HDAC1 and HDAC3 expression correlate with estrogen and progesterone receptor expression pointing to HDAC1 as an independent prognostic marker (Krusche et al., 2005). In a tissue microarray representing 44 categories of malignant and borderline mesenchymal tumors, HDAC2 is highly expressed compared to HDAC1 indicating that HDAC2 more likely contributes to the pathogenesis of the disease (Pacheco and Nielsen, 2012). HDAC2 is also aberrantly expressed in lung cancer tissues (Jung et al., 2012). Taken together, these studies point to overexpression of class I HDACs, in particular HDAC1, as a cancer marker associated with poor prognosis. However, the expression and, therefore, the potential contribution of other HDACs, were not evaluated. In contrast to other members of the class I subfamily, HDAC8 expression appears to be cancer-type specific. High levels of HDAC8 expression have been reported in childhood neuroblastoma (Oehme et al., 2009). In these patients, HDAC8 expression correlates with advance stage disease, poor prognosis and poor survival.

Mutations of HDACs have also been observed. Somatic mutations of the HDAC2 gene in human epithelial cancers with microsatellite instability have been identified (Ropero et al., 2006). A HDAC2 truncating mutation has been detected in a significant number of investigated cancers with microsatellite instability associated with loss of HDAC2 protein expression. Interestingly, the mutation is shown in functional assays to confer resistance to the anti-proliferative and pro-apoptotic effects of HDAC inhibitors (Ropero et al., 2006).

There are hundreds of studies using established cell lines that link class I HDACs with cancer. siRNA-mediated knockdown of HDAC1 and HDAC3 in HeLa cells results in inhibition of cell proliferation, whereas knockdown of HDAC4 and HDAC7 has no effect on cell numbers (Glaser et al., 2003). HDAC1 silencing also results in cell cycle arrest, cell growth inhibition, and induction of apoptosis in osteosarcoma and breast cancer cells (Senese et al., 2007). In colon cancer cells HDAC1 and HDAC2 silencing suppress cell growth (Weichert et al., 2008). HDAC1 overexpression leads to an increase in proliferation and to an undifferentiated phenotype in cultured prostate cancer cells (Halkidou et al., 2004). HDAC1 knockdown in neuroblastoma cells induces the expression of the urokinase plasminogen activator (uPA) leading to an increase in the invasive capacity of the cells in vitro. A similar effect has been observed after cell treatment with the unselective HDIs TSA, butyrate and scriptaid (Pulukuri et al., 2007). Knockdown of both HDAC1 and HDAC2, but not HDAC3, HDAC6 and HDAC8 sensitizes chronic lymphocytic leukemia (CLL) cells for TRAIL-induced apoptosis (Inoue et al., 2006). In breast cancer cell lines, overexpression of HDAC1, HDAC6 or HDAC8 increases cell invasion and MMP9 (Park et al., 2011). HDAC1 is also up-regulated in a subset of human liver cancer cell lines.

Its inactivation results in the regression of tumor cell growth, the induction of autophagic cell death and the increase of p21 and p27 gene expression (Xie et al., 2012). In lung cancer, HDAC1 has been found to be a target of miR-449a. The down-regulation of miR-449a correlates with the up-regulation of HDAC1 (Jeon et al., 2012).

HDAC2 silencing by siRNA in cervical cancer cells causes a differentiated phenotype and an increase in apoptosis associated with the induction of p21Cip1/WAF1 [60]. In breast cancer cells, HDAC2 knockdown increases p53 DNA binding activity that correlates with a block in cell proliferation and the induction of cellular senescence (Harms and Chen, 2007). In human lung cancer cell lines, HDAC2 inactivation results in the induction of apoptosis via p53 and Bax activation. Sustained suppression of HDAC2 in A549 lung cancer cells attenuates the tumorigenic properties of the cells both in vitro and in vivo (Jung et al., 2012). In acute promyelocytic leukemia cells, HDAC3 is recruited to target promoters by PML-RARA, a component of the N-CoR repressor complex, to repress transcription. Knockdown of HDAC3 in these cells restores expression of retinoic acid dependent genes (Atsumi et al., 2006). The AML-1-ETO fusion protein recruits HDAC1, HDAC2 and HDAC3 via ETO to repress transcription of leukemic cells (Amann et al., 2001). HDAC8 knockdown leads to the inhibition of cell proliferation in lung, colon and cervical cancer cell lines (Vannini et al., 2004). The HDAC8 specific inhibitor, PCI-34051 selectively induces apoptosis in T-cell derived lymphoma and leukemic cells, but not in solid cancer cell lines (Balasubramanian et al., 2008). In childhood neuroblastoma cells, knockdown of HDAC8 results in inhibition of proliferation, reduced clonogenic growth, cell cycle arrest and differentiation (Oehme et al., 2009).

3.2. Class II HDACs

3.2.1. Class IIa HDACs

Classically, the study of the role of HDACs in cancer has been focused on the potential contribution of members of the class I HDACs subfamily. However, in recent years class IIa HDACs have started to be linked to several types of cancer. In contrast to class I HDACs, some members of the class IIa subfamily seem to exert a dual role in cancer.

HDAC4 in conjunction with HDAC9 and SIRT5 are found to be overexpressed in patients with Waldenström's macroglobulinemia (Sun et al., 2011). HDAC4 expression is also upregulated in breast cancer samples compared with renal, bladder and colorectal cancer (Ozdogan et al., 2006). However, HDAC4 has not only been reported to be over-represented in cancer. Several studies demonstrate that HDAC4 dysfunction and downregulation are associated with cancer development. On a genome-wide approach, HDAC4 homozygous deletion was observed in melanoma cell lines (Stark and Hayward, 2007). HDAC4 mutations have also been found in breast cancer (Sjoberg et al., 2006).

HDAC5 and HDAC9 seem to be valuable markers for medulloblastoma risk stratification. Both HDACs are over-represented in high-risk medulloblastoma patients, demonstrating a clear relationship between their expression and poor survival (Milde et al., 2010). Knockdown by siRNA of

HDAC5 and HDAC9 in medulloblastoma cells is sufficient to promote cell death (Milde et al., 2010), although the molecular mechanism of their participation in this type of cancer is yet to be identified. HDAC5 is also aberrantly expressed in hepatocellular carcinoma (HCC) together with HDAC3. Their overexpression is correlated with the copy number gains in HCC (Lachenmayer et al., 2012). Treatment with the HDAC inhibitor panobinostat is found to be highly efficient in preclinical models of HCC, confirming the importance of these HDACs in HCC progression (Lachenmayer et al., 2012).

High levels of cytoplasmic HDAC7 have been reported in pancreatic cancer patients (Ouaisi et al., 2008; Weichert, 2009). Similarly, in children with acute lymphoblastic leukemia (ALL), high levels of HDAC7 and HDAC9 expression are associated with poor prognosis (Moreno et al., 2010). Skov and colleagues have reported HDAC7 to be significantly downregulated in myeloproliferative neoplasms (Skov et al., 2012).

Over-representation of HDAC9 has been reported in cervical cancer (Choi et al., 2007). Likewise, the upregulated expression of HDAC9 is associated with poor survival in medulloblastoma patients (Milde et al., 2010) and in childhood acute lymphoblastic leukemia (ALL) patients (Moreno et al., 2010). Overexpressed levels of this histone deacetylase also occur in Waldenstrom's macroglobulinemia patients (Sun et al., 2011) and in classical Philadelphia-negative chronic myeloproliferative neoplasm patients (Skov et al., 2012). Furthermore, HDAC9 is downregulated in glioblastoma relative to low-grade astrocytoma and normal brain (Lucio-Eterovic et al., 2008).

Several studies using cell lines have established a link between class IIa HDACs expression and cancer. These studies indicate that depending on the cellular context, class IIa HDACs can act either as pro-proliferative factors or as tumor suppressors. For example, HDAC4 can either positively or negatively regulate the expression of the cyclin-dependent kinase (CDK) inhibitor p21WAF/Cip1 (Mottet et al., 2009); In the proliferative region of the colonic crypts, HDAC4 interacts with the transcription factor SP1, leading to repression of p21WAF1/Cip1 and increased cell proliferation (Mottet et al., 2009; Wilson et al., 2008). In contrast, after DNA damage, HDAC4 is recruited by p53 to induce p21 expression, promoting DNA repair and resulting in a block in cell proliferation (Mottet et al., 2009). In ovarian cancer cells that are resistant to platinum treatment, HDAC4 is overexpressed and participates in cancer cell survival by deacetylating the transcription factor STAT1 (Stronach et al., 2011). HDAC4 activity is also important in ovarian clear cell carcinoma (CCC). Under hypoxic conditions, the action of the complex composed of HIF2 α /Sp1/HDAC4 leads to the loss of the pro-coagulant activity of CCCs (Koizume et al., 2012). HDAC4 has also been shown to help prostate cancer cells overcome hypoxic conditions by stabilizing HIF-1. Binding of HDAC4 to HIF-1 generates a complex that regulates glycolysis and the cytotoxic stress of cell adaptation to hypoxic conditions (Geng et al., 2011).

In cancer cells, HDAC4 has been shown to be a target of miRNAs. In hepatocellular carcinoma (HCC), HDAC4 levels are raised because of the absence of two miRNAs: miR-1 and miR-22 (Datta et al., 2008; Zhang et al., 2010). Accordingly, HDAC4 downregulation in HCC cells reduces their growth rate (Zhang et al., 2010). miR-1 dysfunction and HDAC4

upregulation, have also been found in lung cancer cells (Nasser et al., 2008). In osteosarcoma and colon cancer cells, lack of miR-140, which targets HDAC4, is associated with chemoresistance. In this context, apoptosis is induced by the p53/HDAC4/p21 pathway when miR-140 is reintroduced (Song et al., 2009).

HDAC4 nuclear localization is important in cancer as HDAC inhibitors block its entrance to the nucleus (Kong et al., 2011). In line with this observation, one study reported that oncogenic Ras can promote HDAC4 nuclear accumulation via activation of the ERK1/2 pathway (Zhou et al., 2000). However, it is not known how this might contribute to Ras-mediated transformation.

Additionally, HDAC4 downregulation and cancer progression both occur in chondrosarcoma cells. These cells have lower levels of HDAC4 than normal chondrocytes. Loss of HDAC4 results in an increase in the level of VEGF expression, which is closely related to the angiogenic capacities of the chondrosarcomas (Sun et al., 2009). Another mechanism by which HDAC4 repression can facilitate cancer development operates in prostate cancer cells, where it represses the transcriptional activity of the androgen receptor (AR), mainly through enhanced SUMOylation rather than deacetylation. Endogenous HDAC4 positively regulates the SUMOylation of endogenous AR and suppresses the induction of prostate-specific antigen expression and cell growth. When HDAC4 is downregulated in prostate cancer cells, AR is not repressed and so cell growth and proliferation are induced by androgens (Yang et al., 2011).

In pancreatic cancer, Ishikawa and colleagues found that the marker of poor prognosis for that cancer, oxysterol binding protein-related protein 5, controls the expression of HDAC5 (Ishikawa et al., 2010). The molecular mechanism behind HDAC5 participation in pancreatic cancer seems to be based on its capacity to regulate PTEN expression. Several studies have revealed a specific function for HDAC5 in cancer cell growth and proliferation. The first data obtained by overexpression studies showed HDAC5 to be a negative regulator of cell proliferation in several cancer cell lines (Huang et al., 2002). In breast cancer cells, HDAC5 regulates p14 repression in association with TBX3 and induces cell proliferation (Yarosh et al., 2008). HDAC5 has also been related to invasion and metastatic features of gastric cancer. Recently, Peixoto and colleagues reported another mechanism by which HDAC5 participates in cancer cell growth. They showed that HDAC5 is necessary for replication fork progression in cancer cells, maintaining and assembling the structure of pericentric heterochromatin in cancer cells (Peixoto et al., 2012).

In breast cancer cells, HDAC7 contributes to cell growth through cooperation with ER α in the repression of Reprimo, a cell cycle inhibitor and tumor suppressor gene (Malik et al., 2010). After siRNA-mediated HDAC7 silencing in breast cancer cells, 17- β -estradiol (E2)-mediated repression of Reprimo is blocked, inducing apoptosis (Malik et al., 2010). In this context, Khurana and colleagues have identified additional factors associated with HDAC7 that are involved in regulating the proliferation of breast cancer cells. They reported that HDAC7 is also associated with ACTN4, potentiating ER α transcriptional activity and, in turn, promotes cancer cell proliferation (Khurana et al., 2010). Interestingly, HDAC7 expression was

found to be specifically downregulated by HDI treatment of cancer and normal cell lines (Dokmanovic et al., 2007; Duong et al., 2008). Wen and colleagues addressed the HIF-1-dependent mechanism involved in cancer cell chemoresistance, and identified an HDAC7/HIF-1 complex that represses expression of cyclin D1. They concluded that HIF-1 participation in chemoresistance could be due to the repression of cyclin D1 by the HDAC7/HIF-1 complex (Wen et al., 2010). Accordingly, co-treatment with HDIs and customary chemotherapeutics is sufficient to overcome the drug-resistant phenotype in these cells. In cultured and primary cutaneous T-cell lymphoma (CTCL) cells, treatment with the pan-HDI panobinostat depletes HDAC7 expression, thereby inducing its target gene Nur77 and, in turn, promoting apoptosis (Chen et al., 2009). In addition, the combined treatment of panobinostat and ABT-737, which is a small molecule inhibitor of the anti-apoptotic proteins Bcl-2, Bcl-xL and Bcl-B, has a synergistic effect on the apoptosis of HDI-sensitive and HDI-insensitive cells (Chen et al., 2009).

Treatment of acute myeloid leukemia (AML) cells with HDIs promotes the overexpression of HDAC9 (Bradbury et al., 2005). Yuan and colleagues found ATCD/TRIM29 (ataxia telangiectasia group D-complementing) to be a substrate of HDAC9 (Yuan et al., 2010). Deacetylation of ATDC by HDAC9 changes the ability of ATDC to bind p53 and consequently affects expression of p53-regulated genes resulting in the reversal of the cell proliferation-promoting activity of ATDC (Yuan et al., 2010). Under these circumstances, HDAC9 would act as a tumor suppressor, providing an explanation for the effect of the HDI treatment and HDAC9 downregulation in glioblastomas. However, the question of how HDAC9 contributes to cancer proliferation remains unresolved.

3.2.2. Class IIb HDACs

High levels of HDAC6 expression have been associated with tumorigenesis (Lee et al., 2008; Sakuma et al., 2006). In oral squamous cell carcinoma, significantly higher HDAC6 expression was found in carcinomas versus normal oral squamous tissue, and HDAC6 expression was increased in advanced-stage cancers compared with early stage (Sakuma et al., 2006). In contrast, in breast cancer, HDAC6 expression was associated with better survival and was higher in small tumors, low histologic grade, and in estrogen and progesterone receptor-positive tumors. High levels of HDAC6 mRNA tended to be more responsive to endocrine treatment than those with low levels. HDAC6 may thus serve as a predictive indicator of responsiveness to endocrine treatment and also as a prognostic indicator for breast cancer progression (Zhang et al., 2004). However, in another study analyzing breast cancer tissues, HDAC6 protein expression revealed no significant prognostic differences based on its expression (Saji et al., 2005).

From a mechanistic angle, HDAC6 deacetylates the chaperone Hsp90, resulting in the inhibition of steroid receptor-mediated transcriptional activation. This mechanism has been reported to influence the growth of prostate cancer cells (Gao and Alumkal, 2010) and breast stem cells (Hsieh et al., 2012). Other studies have reported that HDAC6 promotes angiogenesis by regulating the polarization and migration of vascular epithelial cells (Kaluza et al., 2011; Li et al., 2011a,b). In acute lymphoblastic leukemia (ALL) cells, treatment with

tubacin, a HDAC6 specific inhibitor, has an anti-proliferative effect, enhancing the effects of chemotherapy in vitro and in vivo (Aldana-Masangkay et al., 2011). A similar mechanism has been attributed to the induction of apoptosis after treatment of multiple myeloma cells with the HDAC6 inhibitor ACY-1225 (Santo et al., 2012). Moreover, Xu and colleagues demonstrated that HDAC1 and HDAC6 are critical for cytarabine-induced apoptosis in pediatric acute myeloid leukemia after HDI treatment (Xu et al., 2011). Therefore, targeting HDAC6 with specific HDIs should be considered for treating cancer cells, alone or in combination with other chemotherapeutic agents. In neuroblastoma cells, disruption of the HDAC6/HSP90 complex after HDI treatment leads to the activation of a p53-dependent apoptotic pathway and Myc destabilization (Regan et al., 2011). Other studies performed with pan-HDIs have shed light on the various mechanisms regulated by the HDAC6-HSP90 complex that help induce cell death. Li and colleagues showed that p53 and mutated p53 are targets of this complex after tumor cells are treated with SAHA (Li et al., 2011a,b). In addition, As2O3 exerts anti-myeloma effects by inhibiting HDAC6 activity. In this case, NF- κ B inactivation is the mechanism involved in the apoptosis of cancer cells (Qu et al., 2012). The intracellular macrophage migration inhibitory factor (MIF) is also a target of this tumor-activated HDAC6/HSP90 complex. MIF promotes tumor cell survival, and elevated levels of this protein are correlated with tumor aggressiveness and poor prognosis (Schulz et al., 2012).

Few studies have reported a potential role for HDAC10 in cancer. It has been shown that reduced expression of class II HDACs occurs in samples of non-small cell lung carcinoma (NSCLC), and this is correlated with poor prognosis in lung cancer patients. In this study, the low expression level of HDAC10 was the strongest predictor of poor prognosis (Osada et al., 2004). More recently, HDAC10 was linked to gastric cancer and shown to be important in regulating the production of reactive oxygen species (ROS) in this cancer type; Inhibition of HDAC10 causes ROS to accumulate, triggering the intrinsic apoptotic pathway (Lee et al., 2010). HDAC10 expression is stronger in patients with chronic lymphocytic leukemia (CLL), but the levels of most HDAC family enzymes are raised in this pathology, indicating that pan-HDI could yield better results than subtype-specific HDI for the treatment of CLL (Wang et al., 2011).

3.3. Class III HDACs-sirtuins

In recent years growing evidence has also linked sirtuins to cancer. However, as is the case for other HDACs, sirtuins seem to play both a pro-oncogenic as well as a tumor suppressor role in cancer. Similarly to classical HDACs, aberrant expression of several sirtuins is found in many types of cancer. For example, SIRT1 is upregulated in acute myeloid leukemia (AML), prostate cancer and non-melanoma skin cancer (Bradbury et al., 2005; Hida et al., 2007; Huffman et al., 2007; Stunkel et al., 2007), whereas it has been downregulated in colon tumors (Ozdogan et al., 2006). SIRT3 and SIRT7 are upregulated in breast cancer (Ashraf et al., 2006). In contrast, SIRT2 is downregulated in gliomas and gastric carcinoma (Hiratsuka et al., 2003; Inoue et al., 2007a,b). A mutation in

the catalytic domain of SIRT2 that eliminates its enzymatic activity has been described in melanomas (Lennerz et al., 2005). Evidence suggests that SIRT2 acts as a tumor suppressor and that its loss compromises the mitotic checkpoint, contributing to genomic instability and tumorigenesis (Dryden et al., 2003; Hiratsuka et al., 2003; Inoue et al., 2007a,b). SIRT3 has been found to be upregulated or downregulated in different types of breast cancer (Ashraf et al., 2006; Kim et al., 2010). Altered expression of SIRT5 and SIRT6 in cancer has not been reported so far.

Exogenous SIRT1 expression in different cancer cell lines induces P-glycoprotein expression and makes cancer cells resistant to the chemotherapy drug doxorubicin, whereas knock-down of SIRT1 by siRNA partially reverses the drug-resistant phenotype (Chu et al., 2005). SIRT2 appears to act as a tumor suppressor. Its loss compromises the mitotic checkpoint, contributing to genomic instability and tumorigenesis (Dryden et al., 2003; Inoue et al., 2007a,b). SIRT3 can act as a tumor suppressor or promoter. In fibrosarcoma cells, protection against cell death depends on SIRT3 expression (Yang et al., 2007). SIRT3 overexpression rescues p53-induced cell growth arrest in bladder cancer cells (Li et al., 2010). In human colon carcinoma and osteosarcoma cells, SIRT3 suppresses the levels of ROS, HIF-1 α and its target genes. In colon carcinoma cells, SIRT3 knockdown induces tumorigenesis in a xenograft model (Bell et al., 2011). Very recently, SIRT7 has been reported to be a promoter-associated, highly selective H3K18Ac deacetylase that mediates transcriptional repression and stabilizes cancer cell phenotypes. SIRT7 depletion reduces the tumorigenicity of human cancer cell xenografts in mice (Barber et al., 2012).

3.4. Class IV HDACs

Very few studies have reported a potential role for HDAC11 in cancer. HDAC11 has been found to be involved in Hodgkin lymphoma (HL). Small interfering RNAs (siRNAs) that selectively inhibit HDAC11 expression induced apoptosis in HL cell lines and boost the production of tumor necrosis- α (TNF- α) and IL-17 in the supernatants of HL cells (Buglio et al., 2011). Aberrant expression of HDAC11 has been reported in other hematopoietic cell malignancies. For example, Philadelphia-negative chronic myeloproliferative neoplasms (CMPNs) present high levels of HDAC11. Treatment with HDIs reduces splenomegaly and other metabolic symptoms observed in CMPNs patients. However, whether the effects of HDIs in these patients arise from their anti-inflammatory and immunomodulating capacities or from their anti-proliferative and pro-apoptotic potential remains to be established (Skov et al., 2012).

4. Perspectives

The existence of 18 human HDACs raises the idea that, although some share the same biological functions, they may also exert highly specific roles in particular tissues under both normal and pathological situations. Indeed, gene deletion in mice for individual HDACs has revealed that they exert highly specific biological functions. Since the discovery of the

first mammalian HDAC in 1996 more than 3,000 manuscripts have reported a link between HDACs and cancer. Indeed, it is broadly accepted that the expression of HDACs is deregulated in many cancer types. However, we are still far from understanding the contribution of individual HDACs in cancer. A vast number of the studies linking the aberrant expression of HDACs with cancer have involved the use of established cancer cell lines. These studies have shed light on the mechanisms of action of HDACs and HDIs in the disease, but in some cases the findings may not correlate with their real function in cancer patients. Importantly, the specific targets of individual HDACs in vivo and the question of whether they are also deregulated in cancer remain to be determined. In this context, the contribution of specific HDACs in different cancer types is of critical importance and needs to be carefully examined. Given the current state of research and its embeddedness in the “ultra-sequencing” era, it will soon be possible to draw an accurate, global picture of the expression and/or mutations for all HDAC isoforms in a given cancer. This will allow for the design and development of HDAC isoform-specific HDIs and other potential therapeutic agents. Solving these challenges will definitively open new scientific horizons in the HDAC field, with an impact on the future therapeutic approaches to treat cancer patients in a more selective and personalized manner.

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