Review Article

Multifaceted Influence of Histone Deacetylases on DNA Damage Repair: Implications for Hepatocellular Carcinoma

Gan Du[1,2#], Ruizhe Yang[1,2#], Jianguo Qiu[3*] and Jie Xia[1*]

[1]Key Laboratory of Molecular Biology on Infectious Diseases, Ministry of Education, Chongqing Medical University, Chongqing, China; [2]The First Clinical College, Chongqing Medical University, Chongqing, China; [3]Department of Hepatobiliary Surgery, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China.

Abstract

Hepatocellular carcinoma (HCC) is one of the most commonly diagnosed cancers and a leading cause of cancer-related mortality worldwide, but its pathogenesis remains largely unknown. Nevertheless, genomic instability has been recognized as one of the facilitating characteristics of cancer hallmarks that expedites the acquisition of genetic diversity. Genomic instability is associated with a greater tendency to accumulate DNA damage and tumor-specific DNA repair defects, which gives rise to gene mutations and chromosomal damage and causes oncogenic transformation and tumor progression. Histone deacetylases (HDACs) have been shown to impair a variety of cellular processes of genome stability, including the regulation of DNA damage and repair, reactive oxygen species generation and elimination, and progression to mitosis. In this review, we provide an overview of the role of HDAC in the different aspects of DNA repair and genome instability in HCC as well as the current progress on the development of HDAC-specific inhibitors as new cancer therapies.

Citation of this article: Du G, Yang R, Qiu J, Xia J. Multifaceted Influence of Histone Deacetylases on DNA Damage Repair: Implications for Hepatocellular Carcinoma. J Clin Transl Hepatol 2023;11(1):231–243. doi: 10.14218/JCTH. 2022.00079.

Introduction

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and the third leading cause of cancer-related mortality worldwide. According to the World Health Organization's estimation, 905,677 new liver cancer cases and 830,180 affected individuals died in 2020. The 5-year survival rate of HCC is 18%, indicating poor prognosis and limited available treatments. Hepatocarcinogenesis and the development of HCC are complex processes with multiple risk factors, including chronic infection with hepatitis B or C viruses (HBV or HCV, respectively), alcoholism, and exposure to dietary aflatoxin. HCC development involves constant inflammation, causing hepatocyte necrosis and regeneration, which is accompanied by fibrotic generation. As a result of genovariation in passengers, driver genes, and epigenetic modifications, HCC exhibits great molecular heterogeneity.[1]

Genomic instability, which expedites the acquisition of genetic diversity, acts as a facilitating characteristic of cancer hallmarks. Genomic instability is associated with a greater tendency to accumulate DNA damage, which gives rise to gene mutations and chromosomal damage and causes oncogenic transformation and tumor progression.[2] More than 10,000 genes have been detected as significantly mutated genes in HCC, and 26 genes were altered most frequently, such as TP53, CTNNB1, and AXIN1.[3] The high frequency of mutability caused by DNA damage leads to the selective advantage of subclones of cells in tumor tissue. DNA repair pathways, accounting for cell viability by annealing double-strand break (DSB) sites, are deemed a basic origin of resistance to chemotherapy and radiation therapy. In minute DNA repair pathways, DNA repair inhibitor administration needs to be concentrated on select patients with particular DNA mutations. For example, olaparib possesses precise treatment potential for DNA damage response (DDR)-mutated HCC.[4] Taken together, these results emphasize multiple functions of HCC attained from gene mutation and genomic instability.

Histone deacetylases (HDACs) have been shown to impair a variety of cellular processes of genome stability, including the regulation of DNA damage and repair, reactive oxygen species (ROS) generation and elimination, and progression to mitosis. Targeting genome integrity in rapidly cycling cells has always been a preferred strategy in cancer therapy;[5] in this review, we focus on the different aspects of genome instability induced by pharmacological inhibition of HDACs. Here, we illustrate the main processes of DNA
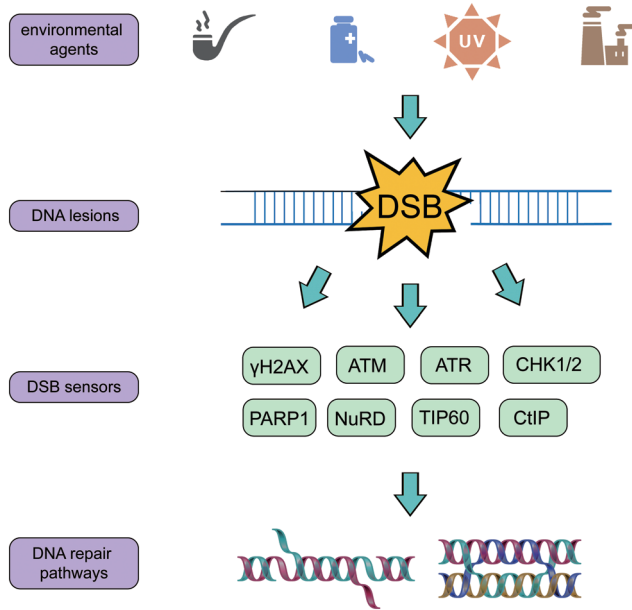
Fig. 1. Model of DDR. DNA damage can be produced by reactive oxygen compounds arising through redox-cycling events involving environmental toxic agents, including tobacco products, chemical drugs, ultraviolet exposure, industrial exhaust pollution, etc. The presence of a lesion in the DNA can block genome replication and transcription. DSB sensors, such as ATM/ATR, CHK1/2 and γH2AX, can recognize chemically and physically derived DNA lesions and recruit various DNA repair factors to initiate DNA repair pathways. DSB, double strand break; ATM, ataxia-telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; CHK1/2, checkpoint kinase 1/2.

repair and epigenetic modification presented by deacetylation in HCC and discuss the possible relationship between them, with the intention of proposing a novel therapeutic strategy by integrating DNA repair and HDAC inhibitors for HCC administration.

Different types of DNA repair pathways

DNA impairment, including single-strand breaks, DSBs, bulky adducts, base alkylation, base mismatches, insertions, and deletions, is caused by various environmental agents, such as cigarette smoke, ultraviolet radiation, industrial chemicals,[6] chemotherapy drugs, and intrinsic agents, such as oxygen radicals and metabolites.[7] DSBs are recognized as one of the ultimate roots for DNA instability and mutation and are associated with several specific repair mechanisms (Fig. 1). Homologous recombination (HR) and classical nonhomologous end joining (NHEJ) act as the major errorless repairs of DSBs, while alternative end joints (alt-EJs) and single-strand annealing (SSA) operate as backups of NHEJ and HR.

HR

HR, mainly occurring in the S and G_2 phases, is a highly conservative and faultless mechanism. (Fig. 2) Its repair involves homologous DNA from the sister chromatid, which, when used as a model, avoids possible mistakes. First, the impaired DNA forms 3'-overhang single-stranded DNA (ssDNA), recruiting human C-terminal-binding protein (CtIP) to bind at the DSB sites as an initiation to enable the MRN complex (constituted by MRE11, RAD50 and NBS1) to attain its nuclease activity and to regulate nucleases EXO1 and BLM/

DNA2.[8] CtIP, as a sensor for DNA damage, controls MRN-directed resection.[9] Phosphorylated RPA loading at ssDNA as a bridge is replaced by recombinase RAD51, which orchestrates breast cancer susceptibility protein (BRCA1)-BRCA1-associated RING domain 1 (BARD1), PALB2, and BRCA2 to make up a helical nucleoprotein filament, facilitating sister chromatid involvement.[10] The filament, in order to repair the lesion, may either undergo the synthesis-dependent strand annealing (SDSA) pathway, with engagement of the Holliday junction, or the double-strand break repair (DSBR) pathway, followed by the recruitment of multiple enzymes, such as GEN1, BLM/Top3a/RIM1 and Mus81-Eme1.[11]

NHEJ

A rapid but not sufficiently accurate mechanism compared with HR, NHEJ mainly occurs in G_1 phase, which connects broken DSBs with randomly synthesized nucleobases. (Fig. 3) Ku (Ku7080 heterodimer) first combines with DSBs as a loading protein to recruit DNA-dependent protein kinase catalytic subunit (DNA-PKcs).[11] DNA-PKcs and Ku together constitute the Ku/DNA-PKcs complex as DNA-dependent protein kinase (DNA-PK).[12] DNA-PKcs undergoes autophosphorylation and then recruits and phosphorylates Artemis.[13] Phosphorylated Artemis gains its DNA-PK-dependent 5' and 3' endonuclease activity and 5' to 3' single-stranded DNA exonuclease activity, enabling it to cut the dissociative DNA end.[14] After that, DNA polymerases (pol), including pol λ, pol μ and terminal deoxynucleotidyl transferase (TdT), are involved in ligation.[15] Otherwise, DNA-PKcs also regulate the essential DNA ligation module Ligase4/X-ray repair cross-complementing 4 (XRCC4)/XLF to stabilize the DNA end structure and fine-tune DNA end ligation.[11]

Alternative end joining

Alt-EJ operates as the backup mechanism of NHEJ. Although alt-EJ can fix DSBs, it will very likely result in large alterations and even the formation of chromosomal translocations.[16] Poly(ADP-ribose) polymerase 1 (PARP1) is involved in sensing DNA damage and binding the end of the DNA.[17] The MRN complex, which is phosphorylated by CtIP and initiates alt-EJ, can be inhibited by Ku competitive combination with DSBs.[18] Alt-EJ can start only if the content of Ku hovers at a relatively low level. The MRN generates 15- to 100-nucleotide 3' overhangs through its endonuclease function, exhibiting the microhomology of DSBs, where the DNA pol θ extends the DNA ends, utilizing the opposite DNA sequence as a replication template.[19] The stable annealing partner is ultimately sealed by DNA ligase I or DNA ligase III (Fig. 4).[20]

SSA

SSA, as a backup to HR, is prone to induce mutations along with severe deletions and translocations.[21] Although HR is the dominant repair mechanism under normal conditions, SSA exerts its function when HR-dependent proteins, RAD51, and its mediator proteins, such as BRCA2 and RAD54, are disrupted.[22] MRN and CtIP are involved in creating the 3 DNA tails, and then EXO1, BLM, and DNA2 extend the tails.[23] Multiple copies of RPA combine with the prolonged DNA end for stability and protection, lessening the formation of secondary structures.[24] After that, RAD52 substitutes for RPA for homology search, strand invasion and annealing.[25] Furthermore, the redundant unannealed flaps are removed by the ERCC1/XPF nuclease, and possible
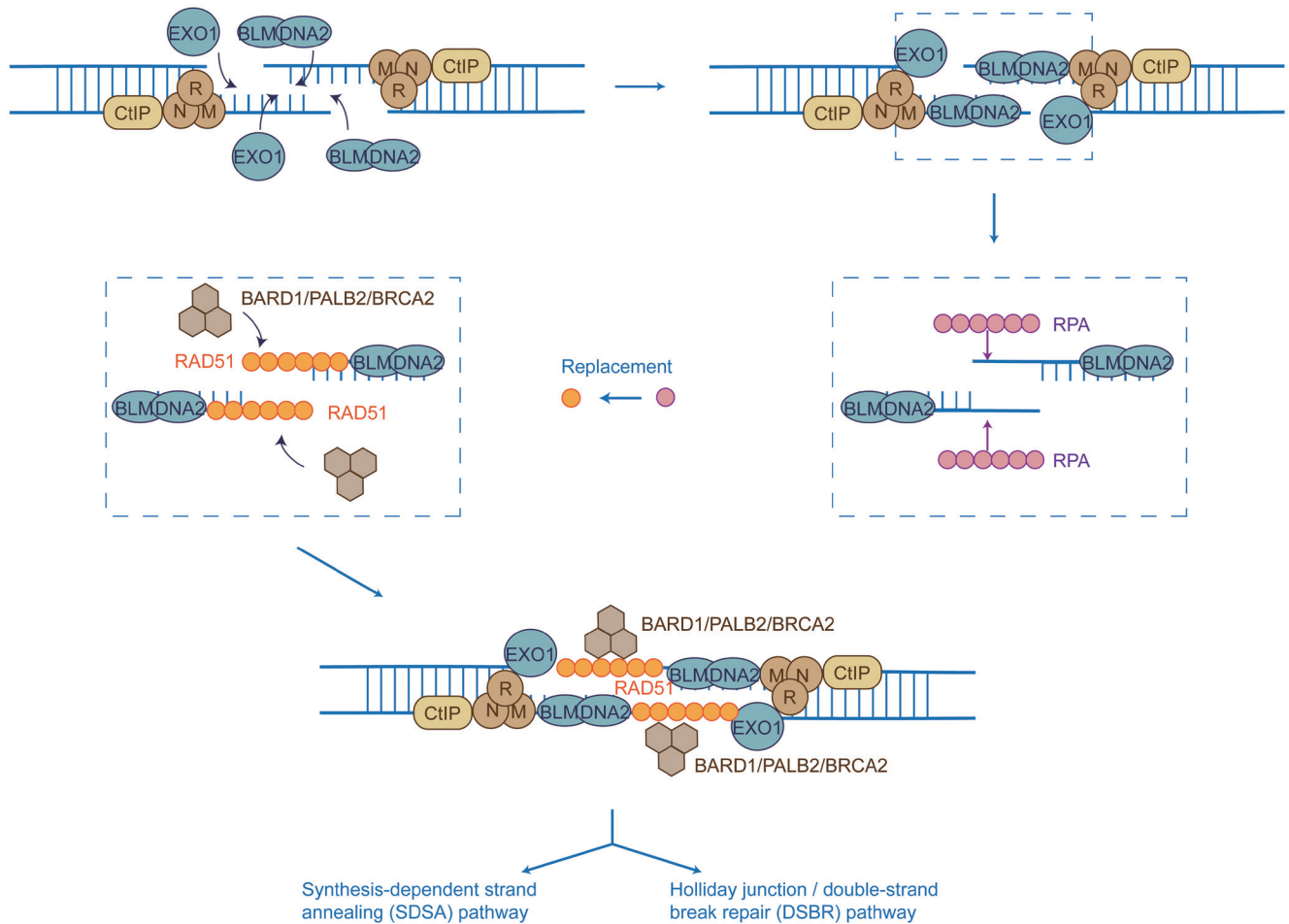
Fig. 2. Overview of homologous recombination. Schematic of DNA double-strand breaks (DSBs) and their repair by homologous recombination. CtIP and MRN bind to the 3'-overhang single-stranded DNA as initiators to regulate nucleases EXO1 and BLM/DNA2, which carry out further resection of DNA and recruit RPA. The recombinase RAD51 replaces phosphorylated RPA and interacts with BARD1, PALB2 and BRCA2, initiating the SDSA pathway or DSBR pathway to repair DNA. DSB, double strand break; CtIP, human C-terminal binding protein; MRN, constituted by MRE11, RAD50 and NBS1; EXO1, Exonuclease 1; BLM, Bloom; DNA2, DNA replication ATP-dependent helicase/nuclease 2; RPA, Replication Protein A; BARD1, BRCA1-associated RING domain 1; PALB2, Partner and Localizer of BRCA2; BRCA2, breast cancer 2; SDSA, synthesis-dependent strand annealing; DSBR, double-strand break repair.

gaps are filled by DNA ligase1 (Fig. 5).[26]

CtIP and MRE11 act as the collective basic molecules of HR, alt-EJ and SSA to start these pathways, whereas NHEJ is initiated by its unique starter, Ku. DNA end resection is of vital importance to pathway choice. The unfavorable environment for resection strengthens the stability of Ku70-Ku80, leading to an inclination of NHEJ. Dislodgement of Ku70–Ku80, as well as the appearance of long-range resections, turns the repair into HR. The error-prone pathways alt-EJ and SSA can hijack the normal HR pathway and generate chromosomal rearrangements.[27] p53-binding protein 1 (53BP1) binds to DNA ends and form irradiation-induced foci, limiting the length of resection and prompting NHEJ.[28] The function of the Shieldin complex is similar to that of 53BP1, blocking DNA end resection and inducing NHEJ.[29] Additionally, phosphorylase ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related protein (ATR) can activate various HR factors, such as MRN, CtIP and EXO1, and enhance DNA end resection-related pathways.[30] Moreover, BRCA2 and RAD51 can overcome the resistance of 53BP1 and the Shieldin complex toward DNA end resection and recover the HR pathway.[31] Additionally, any protein alterations along these pathways can disrupt the dynamic equilibrium. CCCTC-binding factor (CTCF) enhances CtIP recruitment with its N-terminus and ZF domain as the binding site, thus improving the efficiency of HR, as well as alt-EJ and SSA when HR is suppressed.[7] Moreover, CTCF can be modified by PARP1 in a process called PARylation. PARylized CTCF enables the recruitment of BRCA2, further allowing the loading of RAD51 to DSBs.[32] Studies have revealed that the critical DNA pol θ in alt-EJ is often upregulated in cancer tissue but is absent in normal tissue. Pol θ can also bind to RAD51 and inhibit its nucleofilament formation, thus increasing the level to which pol θ can suppress HR.[33]

Classification of HDAC family members and their roles in DNA repair

A total of 18 HDACs remove acetyl groups from histones and nonhistones, which are also called lysine deacetylases or KDACs. These members could be grouped into four types based on their structures. Class I HDACs (HDAC1, HDAC2, HDAC3 and HDAC8) are related to the yeast transcriptional regulator RPD3. Class II HDACs (HDAC4, HDAC5, HDAC6,
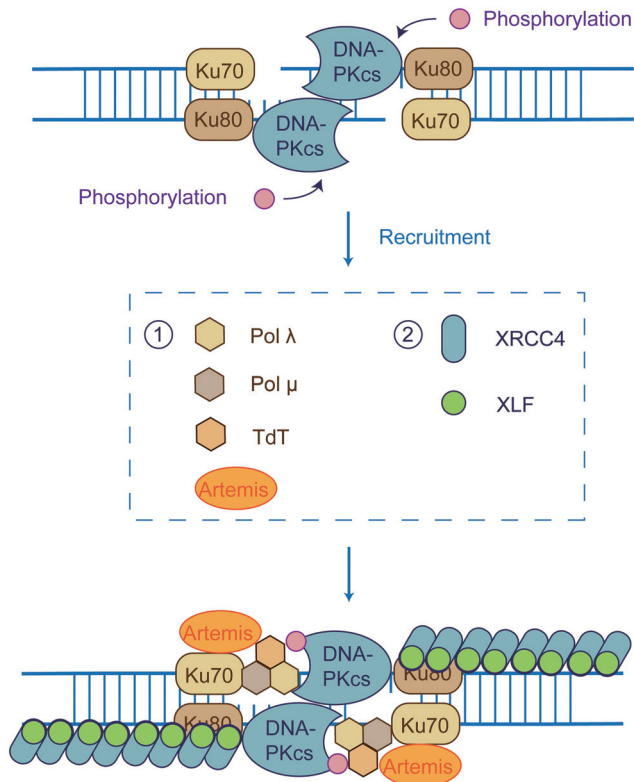
Fig. 3. Overview of non-homologous end joining. The Ku70–Ku80 heterodimer plus DNA-PK catalytic subunit (DNA-PKcs) recruited to a Ku/DNA-PKcs complex binds to DSBs and roles in phosphorylating Artemis and enabling it to cut the dissociative DNA end. Then, the complex improves their subsequent binding by the NHEJ polymerase, nuclease and ligase complexes (pol λ, pol μ, and TdT), which are involved in broken strand repair. XRCC4/XLF are recruited to stabilize the DNA end structure and fine-tune DNA end ligation. DNA-PK, DNA-dependent protein kinase; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; DSB, double strand break; XRCC4, X-ray repair cross-complementing 4; NHEJ. nonhomologous end joining; XLF, XRCC4-like factor.

HDAC7, HDAC9, and HDAC10) share high sequence homology with I1. Class III HDACs share an NAD+-binding catalytic domain. Finally, class IV members only include HDAC11, which is structurally related to both class I and II HDACs.[34,35] Class I, II, and IV are referred to as 'classical' HDACs, whereas class III members are also named sirtuins (SIRTs, including SIRT1-7). Classical HDACs are Zn^{2+}-dependent enzymes harboring a catalytic pocket with a Zn^{2+} ion at its base that can be inhibited by Zn2+-chelating compounds such as hydroxamic acids. Sirtuins (SIRTs) are derived from their homology Saccharomyces cerevisiae gene silent information regulation-2 (Sir2). SIRTs 1, 2, 6 and 7 are located in the nucleus, and SIRTs 1 and 2 can also be found in the cytoplasm. In the mitochondria, SIRTs 3, 4, and 5 can be found.[36] HDACs not only epigenetically modify histone acetylation but also deacetylate various crucial factors associated with different biological processes, including the cell cycle, apoptosis, metabolism, immunity, and ROS production. Specially, an increasing number of studies have shown that HDAC inhibition-related histone acetylation decreases DNA repair and causes DNA damage that is significantly increased in solid tumors. Histone H2AX is a DNA damage sensor and is crucial for DNA integrity.[37] Upon DNA damage, H2AX is phosphorylated at serine 139 to generate γH2AX. This phosphorylation event serves as an anchor for the accumulation of the signaling cascade initiated by DNA damage and requires the activation of DNA-PKcs, ATM, and

ATR. Specifically, the acetylation status of H2AX on Lys5, which is regulated by TIP60 (a histone acetyltransferase) and SIRT1, plays an important role in the formation of γH2AX. The absence of SIRT1 leads to H2AX K5Ac hyperacetylation, lowering DDR levels.[38] The acetylation of histone H4 mainly influences the choice of DNA repair pathway. In response to DNA damage, H4 acetylation follows a rise-fall pattern, which corresponds to rapidly occurring NHEJ and slowly occurring HR.[39] TIP60 mainly contributes to the accumulation of BRCA1 (mediator of HR) and the inhibition of 53BP1 (mediator of NHEJ) at DSB chromatin, while HDACs play the opposite role.[40] The acetylation of histone H3 regulated by HATs/HDACs is required for the binding of BRG1 to γH2AX nucleosomes, and SWI/SNF, γH2AX and H3 acetylation cooperatively act in a feedback activation loop to facilitate DSB repair.[41,42] The regulation of DNA repair pathways by HDACs is summarized in Table 1 and Figure 6.[39,43–96]

Class I HDACs and DNA repair

HDAC1-3 are all highly expressed in HCC, correlating with tumor dedifferentiation and proliferative activity.[97] HDAC1 and HDAC2 participate in the DDR through their location in DSB foci and coupling with accumulated γH2AX, regulation of H3K56 and H4K16 acetylation and requirement for DNA repair, particularly through NHEJ. Cells depleted of HDAC1 and HDAC2 showed DSB repair deficiency, while the DNA damage-induced phosphorylation of the checkpoint kinases CHK1 and CHK2 and the tumor suppressor p53 was higher and more sustained. Moreover, HDAC1/2 inhibition caused the NHEJ factors Ku70/80 and XRCC4 to show enhanced association with DSB sites.[39] In addition to NHEJ, HDAC1, and HDAC2 also participate in the HR pathway through miR-182-related RAD51 regulation. Overexpression of miR-182 decreases RAD51, whereas HDAC1/2 can be recruited to the promoter of miR-182 to diminish its expression, thus promoting HR.[44] In HCC tissue, miR-182 suppresses forkhead box protein (FOXO) 3a and activates the Wnt/β-catenin pathway, enhancing the progression and metastasis of tumor cells.[45]

TIP60 binds to H3K9me3 and transform it to H3K9ac, which acts as a symbol of active transcription, boosting tumor-related gene transcription, including cell cycle regulators, DNA damage-related genes and oncogenic genes. HDAC3 can attach to the H3K9ac site and reverse it to methylation, where DNA repair factors are allowed to initiate HR and NHEJ.[46] Meanwhile, inactivation of HDAC3 also leads to the accrued acetylation of a series of sites on H4, such as H4K5/12/16, the accumulation of which precipitates a reduction in heterochromatin and genomic instability. In HCC, HDAC3 did not significantly increase or even decrease, whereas in HCC, with the loss of HDAC3, hepatoma-related pathways such as p53, g-glutamyltranspeptidase 1, and insulin-like growth factor II are all upregulated. 53BP1 and γH2AX also increase, indicating widely appearing DSB foci.[47] Although there are few reports on the regulation of DNA repair by HDAC8, a test about therapy in acute myeloid leukemia with HDAC8 inhibitor reveals that several DNA sensors (pATM, CHEK1 and CHEK2) and DNA repair factors (CtIP, Rad51, and BRCA1 in HR; Ku70 and DNA-PKcs in NHEJ) are all markedly inhibited.[49]

Class II HDACs and DNA repair

HDAC4 is significantly upregulated in liver cancer and can remodel chromatin structure and control protein binding to DNA, thus regulating oncogenes. Knockdown or inhibition of HDAC4 reduces cell viability, the activation of AKT
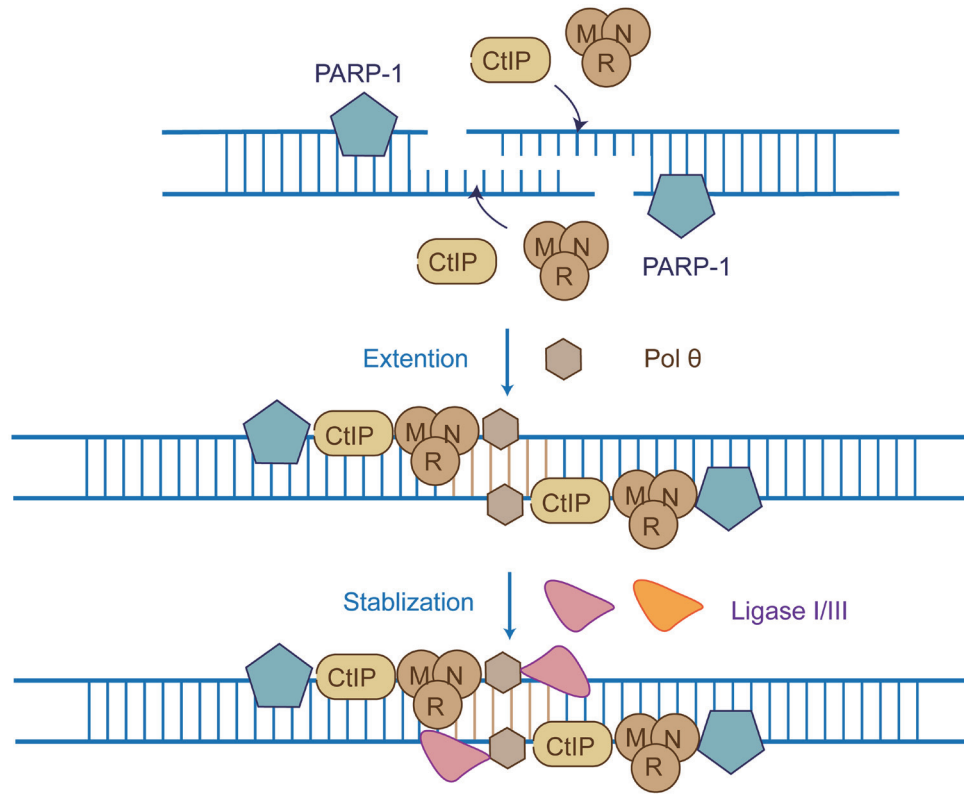
Fig. 4. Overview of alternative end jointing. Alternative end jointing starts when Ku70 and Ku80 remain at low levels. As a sensor, PARP1 binds at the damage site. Then, CtIP phosphorylates MRN to generate 3'-overhangs. DNA pol θ extends the DNA ends, and DNA ligase I/III seals the stable annealing partner. PARP1, poly (ADP-ribose) polymerase 1; CtIP, human C-terminal binding protein; MRN, constituted by MRE11, RAD50 and NBS1.
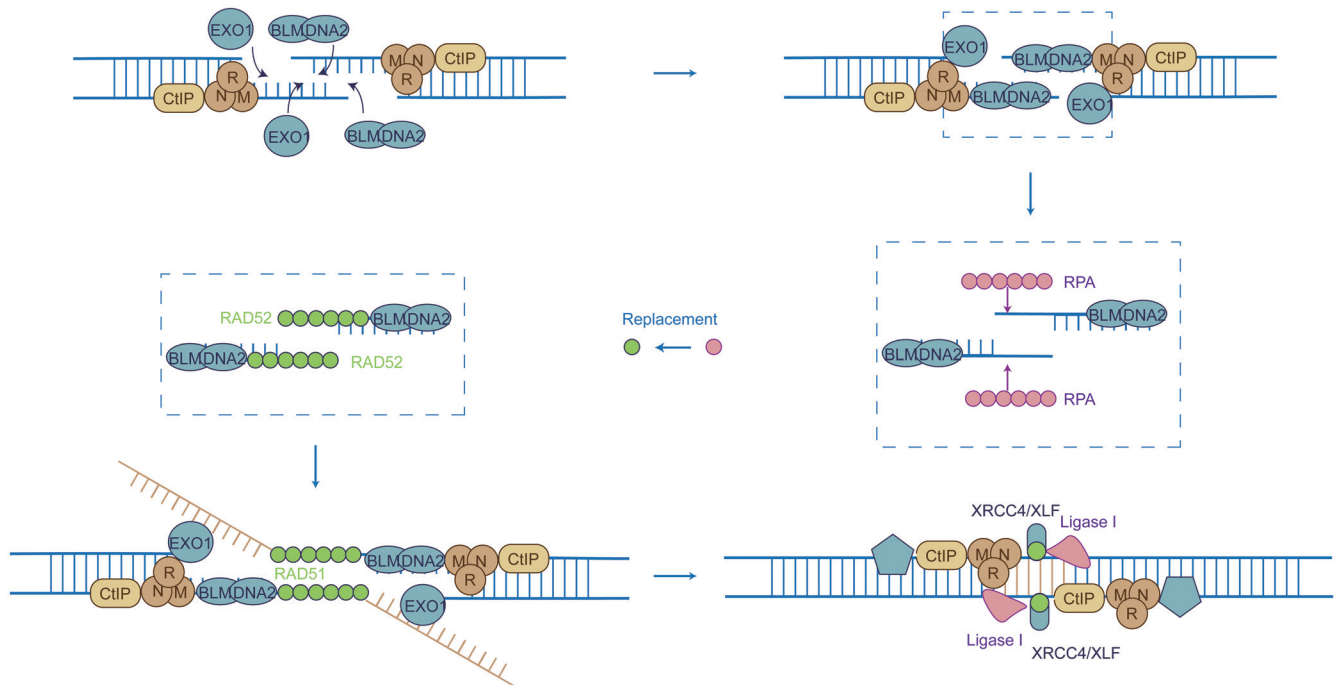
Fig. 5. Overview of single-strand annealing. Single-strand annealing starts when RAD51 and its mediators are disrupted. RAD52 substitutes RPA, generating redundant unannealed flaps, which are removed by the ERCC1/XPF nuclease. RPA, Replication Protein A; ERCC1, Excision repair cross-complementation 1 protein; XPF, Xeroderma pigmentosum complementation group F.

Table 1. Regulative functions of HDACs on DNA repair pathways

| Histone deacetylase		Upregulation	Downregulation	Signal pathway	DNA repair pathway	Relationship with HCC	Reference
Class I	HDAC 1/2	RAD51, FOXO3a	CHK1/2, P53(P), DNA-PKcs(P), Ku70-80, XECC4, Artemis, γH2AX	Wnt/β-catenin pathway↓	NHEJ↓; HR↑	High prevalence of vascular invasion	39,43–45
	HDAC3	DNA repair factors	53BP1, γH2AX	Hepatoma-related pathways (P53, g-glutamyltranspeptidase1, insulin-like growth factor II) ↓	NHEJ↑; HR↑	Suppressing tumor-related genes transcription	46–48
	HDAC8	CtIP, Rad51, BRCA1, ATM(P), Ku70, DNA-PKcs, CHEK1, CHEK2		ATM pathway↑	NHEJ↑; HR↑		49
Class IIA	HDAC4	AKT, γH2AX, UBC9, RAD51, HIC1, FUF		Ferroptosis pathway↑	HR↑	Increasing cell viability, high metastatic potential	50–53
	HDAC7	STAT3, STAT3(P)	CHK1(P), RAD51	NF-κB pathway↑	HR↓	Promoting tumor cell proliferation and invasion	54–56
	HDAC9				HR↑		57
Class IIB	HDAC6*	SMAR1, Ku70, BAX			NHEJ↑		58–61
	HDAC10	BRCA1			HR↑		62,63
Class III	SIRT1	Ku70, BAX, ATM, RAD51, NBS1, Nibrin, WRN, KAP1, HDAC1, BRG1, FOXL2, XRCC5/6, HIC1, FUF	SHP-1	Wnt/β-catenin pathway↑; Ferroptosis pathway↑	NHEJ↑; HR↑; SSA↑	1.Mediating tumorigenesis and chemoresistance; 2. promoting HCC proliferation	50,53,63–75
	SIRT2	BRCA1-BARD1, RAD51, RAD52	Rad51, Artemis, DNA ligase IV, XRCC4		NHEJ↓; HR↓/↑		76–79
	SIRT3	Ku70, BAX, 53BP1, RAD51, RAD52			NHEJ↑; HR↑		71,78–80
	SIRT6	ATM, PARP1, Rad51, Rad51C, Rad52, NBS1, CHD4, SNF2H, CtIP, NuRD, HP1α, RNA polymerase II	HP1, KDM2A		NHEJ↑; HR↓	1. Increasing transformation and tumor tolerance to cell stress; 2. promoting proliferation, migration, invasion, colony-forming ability and cell apoptosis; 3. downregulating infiltration of CD8 T cells	81–93
	SIRT7	53BP1	DNA methyltransferase 1, SIRT1		NHEJ↑; HR↓		94–96

*SIRT6: HR are downregulated in high-grade serous ovarian carcinomas and upregulated in glioblastoma. P, phosphorylation.
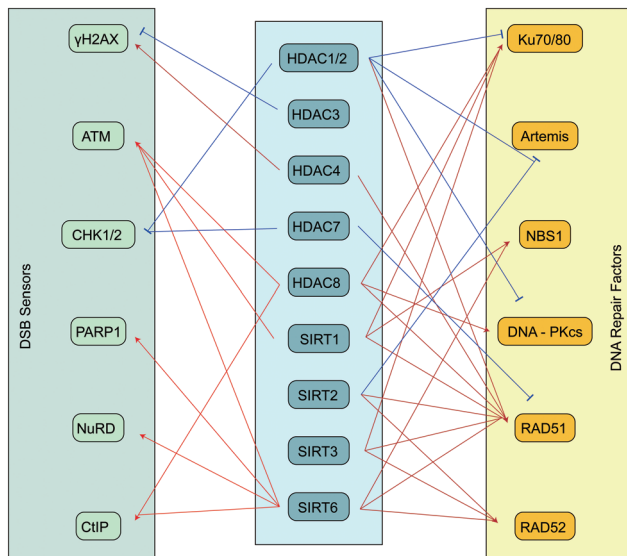
Fig. 6. Schematic diagram of the regulatory network of HDACs on DNA sensors and DNA repair factors.

and the induction of apoptosis. RAD51 and γH2AX are decreased in HDAC4 knockdown HCC cells, indicating that HR repair can be regulated by HDAC4. Moreover, HDAC4 and Rad51 interact with the SUMO-conjugating enzyme Ubc9, and the HDAC4/Ubc9/RAD51 complex can act as a target of radiosensitization for DNA repair in HCC.[53] In addition, HDAC4 can act as a SUMO E3 ligase. HDAC4 interacts with SUMOylation of SIRT1 to form the SIRT1-SUMO-1/HDAC4/Ubc9 complex and combines with hypermethylated in cancer 1 (HIC1), a tumor suppressor gene, to drive deacetylation and SUMOylation of HIC1. SUMOylated HIC1 then enhances its cooperation with MTA1, a component of the NuRD complex, to repress the transcription of target genes that favor the DNA repair process.[50]

HDAC7 has a repressive role by forming a complex with signal transducer and activator of transcription 3 (STAT3) and Tip60 to inhibit gene expression, which is related to STAT3-mediated transactivation. Tip60 binds with HDAC7 on its N-terminal zinc finger-containing region and is essential for its repressive function.[54] Activated STAT3 further decreases the phosphorylation of checkpoint kinase 1 (CHK1), suppressing the intra-S phase cell cycle checkpoint activation. Phosphorylation deficiency of CHK1 impairs RAD51 nucleation, thus curtailing HR.[56]

Ku70 and scaffold matrix attachment region-binding protein 1 (SMAR1) aggregate at DSB sites. SMAR1 connects Ku70 and HDAC6 to form a triple complex to induce deacetylation of Ku70, promoting Ku70 binding to DSB sites.[58] The combination of Ku70 and BCL2-associated X protein (BAX) depends on the deacetylation of Ku70 with HDAC6, the loss of which leads to the release of BAX, resulting in apoptosis.[59] In high-grade serous ovarian carcinomas, HDAC6 removes acetylation from H4K12 and H4K16, inducing HR deficiency, which increases the sensitivity to chemotherapy.[60] In contrast, HDAC6 activates its downstream factor, Sp1, to upregulate RAD51, CHEK1, EXO1, RAD54L, and GEN1, promoting HR repair in glioblastoma cells.[61]

As an epistatic gene of BRCA1, HDAC10 can compensate for the loss of BRCA1 in the cell repair process and reduce the appearance of DSBs. Although BRCA1 is lost, ovarian carcinoma cells can still exert their repair function by HDAC10, while loss of HDAC10 worsens the DSB repair defect. A study utilizing a tissue culture-based homology-

directed repair assay revealed that depletion of HDAC9 or HDAC10 specifically inhibits the HR pathway in HeLa cells.[57]

Sirtuins and DNA repair

The influence of SIRTs on cell viability can be attributed to their protection of telomeres and the activation of all SIRTs instead of only one SIRT, resulting in protection against metabolic disorders, age-related diseases and stem cell failure. The defensive function is based on NAD$^+$ precursors, such as nicotinamide mononucleotide (NMN). During cell damage, the level of NAD$^+$ is significantly decreased, which worsens telomere dysfunction. NMN helps to defend against liver fibrosis at the DNA level, which stabilizes telomeres together with SIRT1. In addition, SIRT6 can also combine with telomeres and deacetylate its H3K9 and H3K56 sites, which is essential for telomere capping. When telomeres are established, the repression of SIRTs is achieved by the DNA damage response and p53. During p53-dependent regulation, nonmitochondrial SIRTs are suppressed at the translational level, while mitochondrial SIRTs are transcriptionally regulated. The upstream factors of nonmitochondrial SIRTs are all highly selective. Nonmitochondrial SIRTs are affected by PGC-1α and PGC-1β, whereas mitochondrial SIRTs are regulated by miR-34a, 26a, and 145.[98]

SIRT1

SIRT1, the most thoroughly studied sirtuin, is involved in a host of biological behaviors in the liver, such as lipid metabolism, oxidative stress and inflammation. SIRT1 acts as a stress sensor and couples with cellular metabolic/energy status, regulating transcription factors such as ChREBP, SREBP-1c, PPARα, PGC-1α, NF-κB, WNT, FOXO family, p53, and p65. When confronted with damage triggers, SIRT1 deacetylates downstream proteins to preserve cell viability. Nonetheless, if extreme damage occurs, SIRT1 helps cells proceed through the apoptosis pathway. Some factors, such as alcohol consumption and a high-fat diet, can impair the function of SIRT1, leading to alcoholic and nonalcoholic fatty liver diseases.[99,100] In liver tissue with ischemic injury, SIRT1 expression and activity are upregulated to compensate for injury. This function can be abrogated by SIRT1 knockdown.[101] In HCC tissues, SIRT1 mainly acts as an oncogene that mediates tumorigenesis and chemoresistance, promoting HCC proliferation and indicating poor prognosis in patients with liver cancer.[63]

SIRT1 has been proven to participate in both the NHEJ and HR repair pathways via its nonhistone protein deacetylation function. Deacetylation of Ku70 blocks the migration of the proapoptotic factor BAX toward mitochondria, thus preventing mitochondrial apoptosis and giving rise to the NHEJ repair pathway with Ku70.[65] SIRT1 is the most important deacetylase of Ku70, and inhibition of SIRT1 enhances Ku70 acetylation, thereby directly obstructing the NHEJ repair pathway.[64] SIRT1 can also remove acetylation from KAP1, thus stabilizing the interaction between KAP1 and 53BP1 to respond to DSBs and promoting NHEJ.[69] In addition, SIRT1 deacetylates HDAC1 and mediates the NHEJ repair function.[70] Finally, SIRT1 is a crucial mediator of the SIRT1-FOXL2-XRCC5/6 axis. FOXL2, as a modulator between NHEJ and HR, can be deacetylated by SIRT1 on the lysine 124 residue to release XRCC5/6. This process is fulfilled by the recruitment of SIRT1 to the nucleus when DSBs occur. Freed XRCC5/6 constitutes the Ku complex to allow the NHEJ pathway and compete for HR.[75]

Inactivated SIRT1 causes a reduction in RAD51, indicating that the HR repair pathway is also regulated by SIR1.[66]

At DSB sites, SIRT1 recruitment depends on ATM, whereas ATM autophosphorylation is performed and stability is ensured by SIRT1, indicating a cooperative relationship between ATM and SIRT1. SIRT1 promotes HR by deacetylating important proteins, such as NBS1 and Rad51. However, high acetylation levels of NBS1 and Rad51 can conversely downregulate SIRT1 activation. In addition, acetylation on NBS1 can be substituted with phosphorylation by SIRT1, as well as ATM, to promote HR.[67] Another mechanism by which SIRT1 regulates the HR repair pathway is via BRG1 deacetylation. BRG1 is one of the major components of the SWI/SNF complex and contributes to the cell cycle in HCC.[73] PAR (activated PARP) recruits SIRT1 and BRG1 to DSB sites, where SIRT1 deacetylates BRG1 to release its ATPase activity to loosen the DNA structure, enhancing HR.[72] SIRT1 also deacetylates nibrin and WRN helicase to promote MRN complex generation for HR initiation.[68]

SIRT 2 and 3

As a negative regulator of stress, radiation-induced impairment can be attenuated by SIRT2 depletion-related DSB repair. Depletion of SIRT2 enhances the expression of several DNA repair proteins, including Rad51, Artemis, DNA ligase IV and XRCC4, therefore improving HR and NHEJ efficiency.[76] SIRT2 deacetylates conserve lysine residues of BARD1 to enable BRCA1 binding, thus catalyzing BRCA1-BARD1 heterodimerization to maintain their mutual stability, promoting HR and prohibiting tumorigenesis.[77] SIRT2 and SIRT3 are responsible for recruiting RAD51 to DSB sites and activating RAD52 by deacetylation, and the deacetylated RAD52 participates in RAD51 recruitment. Both RAD51 and RAD52 are responsible for initiating DSB end resection at the early stage of HR, thus maintaining genome integrity and stability.[78] SIRT3 colocalizes with γH2AX and 53BP1. The recruitment of SIRT3 depends on ring finger protein 8 (RNF8), and SIRT3 removes acetylation from H3K56 and attracts 53BP1 to DSB sites to enhance NHEJ.[80]

SIRT6

SIRT6 acts as a longevity gene that wields various functions to retain cell viability in aging cells, such as maintaining genome integrity.[81] In aging cells, SIRT6 and NHEJ are downregulated, while short-term calorie restriction is associated with increased levels of DNA-PK and SIRT6 to enhance NHEJ.[89] As a DSB sensor for DDR initiation, SIRT6 is located in DSB sites to recruit repair proteins from HR and NHEJ and ATM to fulfill H2AX phosphorylation.[82]

SIRT6 possesses NAD^+-dependent protein deacetylase activity and mono (ADP-ribosyl) transferase activity, acting as a cross point between DNA repair and transcription adjacent to DSB sites to guarantee successful DNA repair. SIRT6 mono-ADP-ribosylates stimulates PARP1 poly-ADP-ribose polymerase activity, which enhances HR repair factors such as Rad51, Rad51C, Rad52, and NBS1 under oxidative stress,[83] SIRT6 translocates to the DNA damage site and displaces HP1 to CHD4 on H3K9. HP1 executes its tumor suppressive and homeostasis regulating function by targeting chromatin activation, which is characterized by H3K9me3. Loss of HP1 renders HCC cells more tolerant to cell stress and increases the possibility of transformation.[90] SIRT6 cooperates with ATM and the chromatin remodeler CHD4 to promote chromatin relaxation and recruit the chromatin remodelers SNF2H and CtIP to the compacted chromatin in HR.[89] The interaction between CHD4 and NuRD is associated with the deacetylation of HDACs and PARP. CHD4/NuRD plays an oncogenic role in EpCAM+ liver cancer

stem cells and in HCC cells and promotes proliferation, migration, invasion, colony-forming ability and cell apoptosis by regulating histone epigenetic status and the DDR.[91] The CHD4/NuRD complex also represses the expression of complements and downregulates the infiltration of CD8 T cells in HCC tissue.[92]

SIRT6 dislodges KDM2A from the chromatin by mono-ADP-ribosylation of the lysine demethylase JHDM1A/KDM2A, resulting in increased H3K36me2 levels. In liver cancer stem cells, KDM2A induces the demethylation of H3K36 in the promoter regions of the transcription factors such as NANOG, SOX4, and OCT4, leading to tumor progression.[93] Furthermore, H3K36me2 promotes H3K9 trimethylation by HP1α binding, leading to the recruitment of RNA polymerase II and NHEJ factors to transiently suppress H3K9 trimethylation.[86] SIRT6 binds with DNA-PK to form a macromolecular complex to activate the DNA-PK catalytic subunit to stabilize DNA-PKcs at chromatin adjacent to an induced site-specific DSB.[87] SIRT6 can interact with Ku80 to enable Ku80 to combine with DNA-PKcs, enhancing DNA-PKcs phosphorylation for efficient NHEJ.[88]

SIRT7

SIRT7 can be mobilized to DSB sites to compact DNA during DNA end-joining. SIRT7 can be recruited by PARP1 to DSB sites to deacetylate H3K18Ac for 53BP1 loading to start NHEJ.[94] Transient upregulation of Dicer releases overloaded SIRT7 from DSBs and prevents its recruitment to maintain the DNA open state, thus promoting NHEJ factors to DSBs to moderately enhance NHEJ.[96] In ribosomal DNA (rDNA), it is necessary to maintain highly compact heterochromatin to inhibit HR between rDNA repeats and protect nucleolar architecture and genomic stability. SIRT7 recruits DNA methyltransferase 1 and SIRT1 to form heterochromatin and avoid HR, while a lack of SIRT7 leads to nucleolar fragmentation, rDNA, and genomic instability.[95]

HDAC and DNA repair inhibitors associated with HCC

DNA repair inhibitors

Faultless DNA repair pathways such as HR and NHEJ in HCC render tumor cells viable after radiation and chemotherapy by stimulating the DNA damage response and avoiding apoptosis. DNA repair factors, such as DNA-PK, ATM, ATR, Ku70/80, and PARP1, contribute to repair progression and induce drug resistance and poor prognosis. Therefore, drugs targeting these factors have been administered in studies and clinical therapies. PARP inhibitors such as olaparib, niraparib and rucaparib have been proven to exert positive functions in patients with BRCA mutant ovarian cancers during phase I and II experiments.[102] Meanwhile, inhibition of DSB recognition, end processing, and DNA ligation processes has been indicated to enhance radiation therapy efficiency. Amid HCC treatment, inhibitors such as olaparib have been proven to significantly reduce malignant tumor phenotypes, such as drug resistance and cancer stem cell survival.

Seventeen PARPs constitute the PARP family, whose functions relate to DSB site recognition and the synthesis of poly (ADP-ribose). PARP1 is recognized as the most researched protein and has been shown to be directly related to HR and NHEJ. Similar to SIRTs, PARP1 exerts its function by relying on NAD^+ substrates to synthesize PARs to target proteins such as PARP itself and other DNA repair factors.[103] PARP1 is significantly upregulated in embryonic stem cells and re-

Table 2. Pharmacological functions of HDAC inhibitor

Classification	HDAC Inhibitors	Target HDACs	Target Proteins/Pathways	Effects on DNA repair	Reference
Cyclic peptides	Romidepsin	HDAC1/2	Erk/cdc25C/cdc2/cyclin B↑; JNK/c-jnk/caspase-3↑	1. Increase acetylation of DNA repair factors (PARP1) to inhibit DNA repair; 2. Decrease the MRP1 transporter to increase intracellular concentration of alkylating agents and lead to the increase of DSBs; 3. Relax chromatin structure and make DNA more susceptible to alkylation	106
Valproic acid	Valproic acid sodium	HDAC1/2/3/5/6	TRAIL↑; caspases 3/9↑; cyclin A/D1↓; p21 and p63↑; MHC class I chain-related molecules↑	Increase radiosensitivity and reduce DSB repair capacity	107
Unclassified	Sodium phenylbutyrate	HDAC1/2/3/4/5/7/8/9	P21$^{WAF1/CIP1}$↑	1. Inhibit HR by mediating changes in chromatin acetylation; 2. Promote DNA repair and survival in normal cells after radiation with lower oxidative stress and TNF-α expression	108,109
Hydroxamates	Panobinostat	HDAC1/2/3/4/5/6/7/8/9/10/11	caspases 4/12↑; Beclin1, Map1LC3B↑; N-cadherin, vimentin, TWIST1, VEGF↓; gankyrin/STAT3/Akt↓	1. Downregulate cyclin E and HR repair pathway genes; 2. Stimulate the activation of DNA damage response through increasing the mitochondrial outer membrane permeability and releasing cytochrome C; 3. Reverse the overexpression of ACTL6A on the cisplatin-induced DNA damage repair to make cells more sensitive to cisplatin	110–112
	Vorinostat	HDAC1/2/3/5/6/8/9/10/11	cell recognition↑; topoisomerase IIα↑	1. Strongly inhibit NHEJ pathway after radiation and enhance tumor radioresponse by antiproliferative growth inhibition; 2. Lead to structural chromosomal aberrations, oxidative DNA strand breaks, DNA hypomethylation, and apoptosis; 3. Suppressed DNA DSB repair proteins (RAD50, MRE11) in cancer but not normal cells	113–115

sidual liver tumors after sorafenib treatment but gradually decreases during hepatic differentiation, which is critical to HCC stem cell pluripotency, residual tumor survival, and the potential of HCC sorafenib treatment resistance.

HDAC inhibitors

HDACs participate in various cellular behaviors of tumorigenesis and are associated with apoptosis, proliferation, metastasis, and senescence of cancer cells by targeting various signaling pathways and DNA-binding sites. Thus, HDAC inhibitors present a promising clinical measure for the treatment of malignant carcinoma, and several HDAC inhibitors have already been approved for hematologic malignancies and lymphomas since 2006. Moreover, the success of the clinical trial of chidaniline in the treatment of hormone receptor-positive breast cancer brought new insight into HDAC inhibitors in solid tumor therapy, and now more than 20 clinical studies are ongoing for refractory, advanced and recurrent solid tumors, including HCC.[104]

HDAC inhibitors consist of four major types: hydroxamates, cyclic peptides, aliphatic acids, and benzamides.[105] The pharmacological functions of the major listed HDAC inhibitors are summarized in Table 2.[106–115] Vorinostat, the first FDA-approved HDAC inhibitor, was approved for the treatment of cutaneous T-cell lymphoma (CTCL). Vorinostat belongs to the hydroxamic acid class of inhibitors, and its targets include class I, II, and IV HDACs. Since the efficacy of vorinostat on CTCL was confirmed, many clinical trials were designed to develop it against advanced and refractory tumors, alone or in combination with other inhibitors. Vorinostat obstructs HCC proliferation and promotes apoptosis, similar to autophagy-induced cell death. It also induces NK-cell-dependent cytolysis by cell recognition and directly impedes DNA replication by blocking topoisomerase IIα.[116] Moreover, vorinostat analogs acetylate histones and induce apoptosis by increasing the expression of tumor sup-

pressor miRNAs.[117] A phase I study of sorafenib and vorinostat in advanced HCC revealed that 13 of 16 patients had durable disease control. Although further study was terminated due to the high incidence of toxicities in patients, the efficacy of SAHA in the treatment of HCC deserves further exploration.[118]

Subsequent to vorinostat, the cyclic peptide romidepsin was approved by the FDA in 2009 to treat CTCL. Romidepsin is a natural compound that specifically inhibits HDAC1 and HDAC2. It was reported to inhibit HCC cells by activating both the Erk/cdc25C/cdc2/cyclin B pathway and the JNK/c-jnk/caspase-3 pathway, leading to G2/M phase arrest and cell apoptosis, respectively.[119] Although there are no clinical trials about romidepsin in HCC therapy, several phase I/II studies of romidepsin alone or combined with other inhibitors to treat different solid tumors are ongoing (NCT01537744, NCT01638533, NCT01302808, NCT02393794, etc.).

Valproic acid sodium (VPA), which mainly inhibits HDAC1, is a fatty acid with anticonvulsant properties that can be used to treat epilepsy. It disrupts the formation of single-strand-DNA-RFA nucleofilaments and the activation of ATR and CHK2 by suppressing the recruitment of RPA and ATR interacting protein (ATRIP) to DNA damage sites. VPA has been shown to promote HCC apoptosis by activating tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and caspases 3/9.[120] It also decreases cyclin A and D1 levels and increases p21 and p63 levels to block the cell cycle.[121] Finally, VPA upregulates MHC class I chain-related molecules to avert tumor escape. Its pharmacologic effect mainly involves the regulation of malignant tumor cells, while it has little effect on normal tumors.[120,122]

Panobinostat, a novel broad-spectrum HDAC inhibitor, induces endoplasmic reticulum stress by activating caspases 4/12 and upregulates the autophagy-promoting factors Beclin1 and Map1LC3B, directly leading to apoptosis.[123] Panobinostat also inhibits angiogenesis and metastasis by inhibiting the expression of N-cadherin, vimentin, TWIST1, VEGF and the gankyrin/STAT3/Akt pathway,[124] At present, panobinostat has been approved for the treatment of multiple myeloma by both the US Food and Drug Administration and the European Medicines Agency. Interestingly, it is also expected to be used in HCC treatment, and two phase I clinical trials are ongoing (NCT00823290 and NCT00873002).

Belinostat is a novel pan-HDAC inhibitor that has been developed in various solid tumors and hematologic malignancies. The US FDA granted accelerated approval for belinostat for the treatment of patients with relapsed or refractory peripheral T cell lymphoma (PTCL) due to its high rate of efficacy and low rate of adverse reactions. A phase I/II study of it for patients with unresectable HCC was completed in 2017, and the results showed that the mOS of patients reached 8.9 months and that the median PFS was 2.83 months (NCT00321594).

Resminostat, a dose-dependent selective inhibitor of HDAC1/3/6, has a unique mechanism of action that may expand the therapeutic treatments available to patients with advanced HCC. A SHELTER study provided promising data that combined therapeutic approaches utilizing resminostat was useful for HCC patients who failed sorafenib. Resminostat may counterbalance or even reverse the resistance mechanisms to sorafenib and provide a survival benefit (NCT00943449).[125]

Limitations of HDACi on HCC treatment

Although many HDACis have been developed, the vast majority of them have been proven to have no anticancer effect on solid tumors. In HCC, despite the well-described mechanisms of HDAC inhibition, no phase III clinical trial has been conducted to date. There are several reasons for the lack of phase III trials. First, many clinical trials of HDACi have shown various adverse effects, including bleeding, nausea, neurotoxicity, fatigue, vomiting, anemia, arrhythmia, myocardial hypertrophy, diarrhea, hypophosphatemia, and hyponatremia. Second, not all patients will have the same survival advantage with HDACi; therefore, predictive biomarkers of response and prognostic biomarkers of survival are necessary to design and accumulate patients best suited for clinical studies. Third, although HDACis play a positive role in improving patient survival and symptom control, in most cases, HCC cells develop drug resistance to HDACis, resulting in malignant phenotype regeneration and maintenance.

Conclusions and future perspectives

HCC, as one of the most commonly occurring malignant tumors in the world, is a high-profile medical issue, with an age-adjusted incidence of 10.1 per 100 000 person-years worldwide.[1] The late stage of HCC allows little space for surgical treatment, giving great significance for chemotherapy. Overexpression of HDACs frequently occurs in HCC cells and crucially controls DNA repair and maintenance of the neoplastic phenotype. DNA repair protects cells from deadly DNA lesions. Some key repair factors that undergo acetylation are targets of HDACi. Dysregulation of DNA repair proteins by HDACis might explain the efficacy of HDACis in HCC therapy. An increasing number of HDACi are undergoing preclinical experiments and clinical trials against cancers. Despite the role of HDACi on pathways in other cell processes, such as cell proliferation and metastasis, which have been carefully studied, research on their influence on DNA repair pathways has not yet been carried out. As HDACs participate in DNA repair in multiple pathways, HDACi-mediated dysregulation of DNA repair combined with DNA-damaging chemotherapeutics may overload DNA repair machinery. New approaches using HDACi and DNA repair inhibitors in combination may overcome tumor progression to improve patient survival.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Collection of the data, manuscript writing, drafting the article, imaging and figures (GD, RY), critical revision of the manuscript for important intellectual content (JX, JQ).

References

[1] Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet 2018; 391(10127):1301–1314. doi:10.1016/S0140-6736(18)30010-2, PMID:29 307467.

[2] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646–674. doi:10.1016/j.cell.2011.02.013, PMID:21376230.

[3] Lawrence MS, Stojanov P, Mermel CH, Robinson JT, Garraway LA, Golub TR, *et al*. Discovery and saturation analysis of cancer genes across 21 tumour types. Nature 2014;505(7484):495–501. doi:10.1038/nature12912, PMID:24390350.

[4] Lin J, Shi J, Guo H, Yang X, Jiang Y, Long J, *et al*. Alterations in DNA Damage Repair Genes in Primary Liver Cancer. Clin Cancer Res 2019;25(15):4701–4711. doi:10.1158/1078-0432.CCR-19-0127, PMID:31068370.

[5] Sultana F, Manasa KL, Shaik SP, Bonam SR, Kamal A. Zinc Dependent Histone Deacetylase Inhibitors in Cancer Therapeutics: Recent Update. Curr Med Chem 2019;26(40):7212–7280. doi:10.2174/092986732566618053 0094120, PMID:29852860.

[6] Lord CJ, Ashworth A. The DNA damage response and cancer therapy. Nature 2012;481(7381):287–294. doi:10.1038/nature10760, PMID:22258607.

[7] Hwang SY, Kang MA, Baik CJ, Lee Y, Hang NT, Kim BG, *et al*. CTCF cooperates with CtIP to drive homologous recombination repair of double-strand breaks. Nucleic Acids Res 2019;47(17):9160–9179. doi:10.1093/nar/gkz639, PMID:31340001.

[8] Huertas P. DNA resection in eukaryotes: deciding how to fix the break. Nat Struct Mol Biol 2010;17(1):11–16. doi:10.1038/nsmb.1710, PMID:20051983.

[9] You Z, Bailis JM. DNA damage and decisions: CtIP coordinates DNA repair and cell cycle checkpoints. Trends Cell Biol 2010;20(7):402–409. doi:10.1016/j.tcb.2010.04.002, PMID:20444606.

[10] San Filippo J, Sung P, Klein H. Mechanism of eukaryotic homologous recombination. Annu Rev Biochem 2008;77:229–257. doi:10.1146/annurev.biochem.77.061306.125255, PMID:18275380.

[11] Mladenov E, Magin S, Soni A, Iliakis G. DNA double-strand-break repair in higher eukaryotes and its role in genomic instability and cancer: Cell cycle and proliferation-dependent regulation. Semin Cancer Biol 2016;37-38:51–64. doi:10.1016/j.semcancer.2016.03.003, PMID:27016036.

[12] Hill R, Lee PW. The DNA-dependent protein kinase (DNA-PK): More than just a case of making ends meet? Cell Cycle 2010;9(17):3460–3469. doi:10.4161/cc.9.17.13043, PMID:20855954.

[13] Neal JA, Sugiman-Marangos S, VanderVere-Carozza P, Wagner M, Turchi J, Lees-Miller SP, *et al*. Unraveling the complexities of DNA-dependent protein kinase autophosphorylation. Mol Cell Biol 2014;34(12):2162–2175. doi:10.1128/MCB.01554-13, PMID:24687855.

[14] Chang HH, Lieber MR. Structure-Specific nuclease activities of Artemis and the Artemis: DNA-PKcs complex. Nucleic Acids Res 2016;44(11):4991–4997. doi:10.1093/nar/gkw456, PMID:27198222.

[15] Boubakour-Azzouz I, Bertrand P, Claes A, Lopez BS, Rougeon F. Terminal deoxynucleotidyl transferase requires KU80 and XRCC4 to promote N-addition at non-V(D)J chromosomal breaks in non-lymphoid cells. Nucleic Acids Res 2012;40(17):8381–8391. doi:10.1093/nar/gks585, PMID:22740656.

[16] Rodgers K, McVey M. Error-Prone Repair of DNA Double-Strand Breaks. J Cell Physiol 2016;231(1):15–24. doi:10.1002/jcp.25053, PMID:26033759.

[17] Beck C, Robert I, Reina-San-Martin B, Schreiber V, Dantzer F. Poly(ADP-ribose) polymerases in double-strand break repair: focus on PARP1, PARP2 and PARP3. Exp Cell Res 2014;329(1):18–25. doi:10.1016/j.yexcr.2014.07.003, PMID:25017100.

[18] Makharashvili N, Tubbs AT, Yang SH, Wang H, Barton O, Zhou Y, *et al*. Catalytic and noncatalytic roles of the CtIP endonuclease in double-strand break end resection. Mol Cell 2014;54(6):1022–1033. doi:10.1016/j.molcel.2014.04.011, PMID:24837676.

[19] Chang HHY, Pannunzio NR, Adachi N, Lieber MR. Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat Rev Mol Cell Biol 2017;18(8):495–506. doi:10.1038/nrm.2017.48, PMID:28512351.

[20] Paul K, Wang M, Mladenov E, Bencsik-Theilen A, Bednar T, Wu W, *et al*. DNA ligases I and III cooperate in alternative non-homologous end-joining in vertebrates. PLoS One 2013;8(3):e59505. doi:10.1371/journal.pone.0059505, PMID:23555685.

[21] Weinstock DM, Richardson CA, Elliott B, Jasin M. Modeling oncogenic translocations: distinct roles for double-strand break repair pathways in translocation formation in mammalian cells. DNA Repair (Amst) 2006;5(9-10):1065–1074. doi:10.1016/j.dnarep.2006.05.028, PMID:16815104.

[22] Do AT, Brooks JT, Le Neveu MK, LaRocque JR. Double-strand break repair assays determine pathway choice and structure of gene conversion events in Drosophila melanogaster. G3 (Bethesda) 2014;4(3):425–432. doi:10.1534/g3.113.010074, PMID:24368780.

[23] Daley JM, Kwon Y, Niu H, Sung P. Investigations of homologous recombination pathways and their regulation. Yale J Biol Med 2013;86(4):453–461. PMID:24348209.

[24] Sung P, Krejci L, Van Komen S, Sehorn MG. Rad51 recombinase and recombination mediators. J Biol Chem 2003;278(44):42729–42732. doi:10.1074/jbc.R300027200, PMID:12912992.

[25] Yates LA, Aramayo RJ, Pokhrel N, Caldwell CC, Kaplan JA, Perera RL, *et al*. A structural and dynamic model for the assembly of Replication Protein A on single-stranded DNA. Nat Commun 2018;9(1):5447. doi:10.1038/s41467-018-07883-7, PMID:30575763.

[26] Li S, Lu H, Wang Z, Hu Q, Wang H, Xiang R, *et al*. ERCC1/XPF Is Important for Repair of DNA Double-Strand Breaks Containing Secondary Structures. iScience 2019;16:63–78. doi:10.1016/j.isci.2019.05.017, PMID: 31153042.

[27] Scully R, Panday A, Elango R, Willis NA. DNA double-strand break repair-pathway choice in somatic mammalian cells. Nat Rev Mol Cell Biol 2019; 20(11):698–714. doi:10.1038/s41580-019-0152-0, PMID:31263220.

[28] Bártová E, Legartová S, Dundr M, Suchánková J. A role of the 53BP1 protein in genome protection: structural and functional characteristics of 53BP1-dependent DNA repair. Aging (Albany NY) 2019;11(8):2488–2511. doi:10.18632/aging.101917, PMID:30996128.

[29] Gupta R, Somyajit K, Narita T, Maskey E, Stanlie A, Kremer M, *et al*. DNA Repair Network Analysis Reveals Shieldin as a Key Regulator of NHEJ and PARP Inhibitor Sensitivity. Cell 2018;173(4):972–988.e23. doi:10.1016/j.cell.2018.03.050, PMID:29656893.

[30] Saha J, Wang M, Cucinotta FA. Investigation of switch from ATM to ATR signaling at the sites of DNA damage induced by low and high LET radiation. DNA Repair (Amst) 2013;12(12):1143–1151. doi:10.1016/j.dnarep.2013.10.004, PMID:24238855.

[31] Schwarz B, Friedl AA, Girst S, Dollinger G, Reindl J. Nanoscopic analysis of 53BP1, BRCA1 and Rad51 reveals new insights in temporal progression of DNA-repair and pathway choice. Mutat Res 2019;816-818:111675. doi:10.1016/j.mrfmmm.2019.111675, PMID:31302572.

[32] Hilmi K, Jangal M, Marques M, Zhao T, Saad A, Zhang C, *et al*. CTCF facilitates DNA double-strand break repair by enhancing homologous recombination repair. Sci Adv 2017;3(5):e1601898. doi:10.1126/sciadv.1601898, PMID:28560323.

[33] Ceccaldi R, Liu JC, Amunugama R, Hajdu I, Primack B, Petalcorin MI, *et al*. Homologous-recombination-deficient tumours are dependent on Polθ-mediated repair. Nature 2015;518(7538):258–262. doi:10.1038/nature14184, PMID:25642963.

[34] Roos WP, Krumm A. The multifaceted influence of histone deacetylases on DNA damage signalling and DNA repair. Nucleic Acids Res 2016;44(21):10017–10030. doi:10.1093/nar/gkw922, PMID:27738139.

[35] Bhaskara S. Histone deacetylases 1 and 2 regulate DNA replication and DNA repair: potential targets for genome stability-mechanism-based therapeutics for a subset of cancers. Cell Cycle 2015;14(12):1779–1785. doi:10.1080/15384101.2015.1042634, PMID:25942572.

[36] Pulla VK, Battu MB, Alvala M, Sriram D, Yogeeswari P. Can targeting SIRT-1 to treat type 2 diabetes be a good strategy? A review. Expert Opin Ther Targets 2012;16(8):819–832. doi:10.1517/14728222.2012.703656, PMID: 22762724.

[37] Bonner WM, Redon CE, Dickey JS, Nakamura AJ, Sedelnikova OA, Solier S, *et al*. GammaH2AX and cancer. Nat Rev Cancer 2008;8(12):957–967. doi:10.1038/nrc2523, PMID:19005492.

[38] Yamagata K, Kitabayashi I. Sirt1 physically interacts with Tip60 and negatively regulates Tip60-mediated acetylation of H2AX. Biochem Biophys Res Commun 2009;390(4):1355–1360. doi:10.1016/j.bbrc.2009.10.156, PMID:19895790.

[39] Miller KM, Tjeertes JV, Coates J, Legube G, Polo SE, Britton S, *et al*. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nat Struct Mol Biol 2010;17(9):1144–1151. doi:10.1038/nsmb.1899, PMID:20802485.

[40] Tang J, Cho NW, Cui G, Manion EM, Shanbhag NM, Botuyan MV, *et al*. Acetylation limits 53BP1 association with damaged chromatin to promote homologous recombination. Nat Struct Mol Biol 2013;20(3):317–325. doi:10.1038/nsmb.2499, PMID:23377543.

[41] Lee HS, Park JH, Kim SJ, Kwon SJ, Kwon J. A cooperative activation loop among SWI/SNF, gamma-H2AX and H3 acetylation for DNA double-strand break repair. EMBO J 2010;29(8):1434–1445. doi:10.1038/emboj.2010.27, PMID:20224553.

[42] Hu B, Lin JZ, Yang XB, Sang XT. The roles of mutated SWI/SNF complexes in the initiation and development of hepatocellular carcinoma and its regulatory effect on the immune system: A review. Cell Prolif 2020;53(4):e12791. doi:10.1111/cpr.12791, PMID:32162380.

[43] Wang H, Kohashi K, Yoshizumi T, Okumura Y, Tanaka Y, Shimokawa M, *et al*. Coexpression of SALL4 with HDAC1 and/or HDAC2 is associated with underexpression of PTEN and poor prognosis in patients with hepatocellular carcinoma. Hum Pathol 2017;64:69–75. doi:10.1016/j.humpath.2017.03.007, PMID:28411180.

[44] Lai TH, Ewald B, Zecevic A, Liu C, Sulda M, Papaioannou D, *et al*. HDAC Inhibition Induces MicroRNA-182, which Targets RAD51 and Impairs HR Repair to Sensitize Cells to Sapacitabine in Acute Myelogenous Leukemia. Clin Cancer Res 2016;22(14):3537–3549. doi:10.1158/1078-0432.CCR-15-1063, PMID:26858310.

[45] Cao MQ, You AB, Zhu XD, Zhang W, Zhang YY, Zhang SZ, *et al*. miR-182-5p promotes hepatocellular carcinoma progression by repressing FOX-O3a. J Hematol Oncol 2018;11(1):12. doi:10.1186/s13045-018-0555-y, PMID:29361949.

[46] Ji H, Zhou Y, Zhuang X, Zhu Y, Wu Z, Lu Y, *et al*. HDAC3 Deficiency Promotes Liver Cancer through a Defect in H3K9ac/H3K9me3 Transition. Cancer Res 2019;79(14):3676–3688. doi:10.1158/0008-5472.CAN-18-3767, PMID:31097476.

[47] Bhaskara S, Knutson SK, Jiang G, Chandrasekharan MB, Wilson AJ, Zheng S, *et al*. Hdac3 is essential for the maintenance of chromatin structure and genome stability. Cancer Cell 2010;18(5):436–447. doi:10.1016/j.ccr.2010.10.022, PMID:21075309.

[48] Pokholok DK, Harbison CT, Levine S, Cole M, Hannett NM, Lee TI, *et al*. Genome-wide map of nucleosome acetylation and methylation in yeast. Cell 2005;122(4):517–527. doi:10.1016/j.cell.2005.06.026, PMID:16122420.

[49] Zhang P, Brinton LT, Williams K, Sher S, Orwick S, Tzung-Huei L, *et al*. Targeting DNA Damage Repair Functions of Two Histone Deacetylases, HDAC8 and SIRT6, Sensitizes Acute Myeloid Leukemia to NAMPT Inhibition. Clin Cancer Res 2021;27(8):2352–2366. doi:10.1158/1078-0432.CCR-20-37

24, PMID:33542077.

[50] Dehennaut V, Loison I, Dubuissez M, Nassour J, Abbadie C, Leprince D. DNA double-strand breaks lead to activation of hypermethylated in cancer 1 (HIC1) by SUMOylation to regulate DNA repair. J Biol Chem 2013;288(15):10254–10264. doi:10.1074/jbc.M112.421610, PMID:234 17673.

[51] Xu H, Wang H, Zhao W, Fu S, Li Y, Ni W, *et al*. SUMO1 modification of methyltransferase-like 3 promotes tumor progression via regulating Snail mRNA homeostasis in hepatocellular carcinoma. Theranostics 2020;10(13):5671–5686. doi:10.7150/thno.42539, PMID:32483411.

[52] Zhang X, Du L, Qiao Y, Zhang X, Zheng W, Wu Q, *et al*. Ferroptosis is governed by differential regulation of transcription in liver cancer. Redox Biol 2019;24:101211. doi:10.1016/j.redox.2019.101211, PMID:31108460.

[53] Tsai CL, Liu WL, Hsu FM, Yang PS, Yen RF, Tzen KY, *et al*. Targeting histone deacetylase 4/ubiquitin-conjugating enzyme 9 impairs DNA repair for radiosensitization of hepatocellular carcinoma cells in mice. Hepatology 2018;67(2):586–599. doi:10.1002/hep.29328, PMID:28646552.

[54] Xiao H, Chung J, Kao HY, Yang YC. Tip60 is a co-repressor for STAT3. J Biol Chem 2003;278(13):11197–11204. doi:10.1074/jbc.M210816200, PMID:12551922.

[55] Zhou Q, Tian W, Jiang Z, Huang T, Ge C, Liu T, *et al*. A Positive Feedback Loop of AKR1C3-Mediated Activation of NF-κB and STAT3 Facilitates Proliferation and Metastasis in Hepatocellular Carcinoma. Cancer Res 2021;81(5):1361–1374. doi:10.1158/0008-5472.CAN-20-2480, PMID:333 61392.

[56] McIntosh MT, Koganti S, Boatwright JL, Li X, Spadaro SV, Brantly AC, *et al*. STAT3 imparts BRCAness by impairing homologous recombination repair in Epstein-Barr virus-transformed B lymphocytes. PLoS Pathog 2020;16(10):e1008849. doi:10.1371/journal.ppat.1008849, PMID:33002 095.

[57] Kotian S, Liyanarachchi S, Zelent A, Parvin JD. Histone deacetylases 9 and 10 are required for homologous recombination. J Biol Chem 2011; 286(10):7722–7726. doi:10.1074/jbc.C110.194233, PMID:21247901.

[58] Chaudhary N, Nakka KK, Chavali PL, Bhat J, Chatterjee S, Chattopadhyay S. SMAR1 coordinates HDAC6-induced deacetylation of Ku70 and dictates cell fate upon irradiation. Cell Death Dis 2014;5:e1447. doi:10.1038/cd-dis.2014.397, PMID:25299772.

[59] Subramanian C, Jarzembowski JA, Opipari AW Jr, Castle VP, Kwok RP. HDAC6 deacetylates Ku70 and regulates Ku70-Bax binding in neuroblastoma. Neoplasia 2011;13(8):726–734. doi:10.1593/neo.11558, PMID:21847364.

[60] Thomas SN, Chen L, Liu Y, Höti N, Zhang H. Targeted Proteomic Analyses of Histone H4 Acetylation Changes Associated with Homologous-Recombination-Deficient High-Grade Serous Ovarian Carcinomas. J Proteome Res 2017;16(10):3704–3710. doi:10.1021/acs.jproteome.7b00405, PMID:288 66885.

[61] Yang WB, Wu AC, Hsu TI, Liou JP, Lo WL, Chang KY, *et al*. Histone deacetylase 6 acts upstream of DNA damage response activation to support the survival of glioblastoma cells. Cell Death Dis 2021;12(10):884. doi:10.1038/s41419-021-04182-w, PMID:34584069.

[62] Islam MM, Banerjee T, Packard CZ, Kotian S, Selvendiran K, Cohn DE, *et al*. HDAC10 as a potential therapeutic target in ovarian cancer. Gynecol Oncol 2017;144(3):613–620. doi:10.1016/j.ygyno.2017.01.009, PMID:28073598.

[63] Chen HC, Jeng YM, Yuan RH, Hsu HC, Chen YL. SIRT1 promotes tumorigenesis and resistance to chemotherapy in hepatocellular carcinoma and its expression predicts poor prognosis. Ann Surg Oncol 2012;19(6):2011–2019. doi:10.1245/s10434-011-2159-4, PMID:22146883.

[64] Zhang W, Wu H, Yang M, Ye S, Li L, Zhang H, *et al*. SIRT1 inhibition impairs non-homologous end joining DNA damage repair by increasing Ku70 acetylation in chronic myeloid leukemia cells. Oncotarget 2016;7(12):13538–13550. doi:10.18632/oncotarget.6455, PMID:26646449.

[65] Jeong J, Juhn K, Lee H, Kim SH, Min BH, Lee KM, *et al*. SIRT1 promotes DNA repair activity and deacetylation of Ku70. Exp Mol Med 2007;39(1):8–13. doi:10.1038/emm.2007.2, PMID:17334224.

[66] Yu W, Li L, Wang G, Zhang W, Xu J, Liang A. KU70 Inhibition Impairs Both Non-Homologous End Joining and Homologous Recombination DNA Damage Repair Through SHP-1 Induced Dephosphorylation of SIRT1 in T-Cell Acute Lymphoblastic Leukemia (T-ALL) [corrected]. Cell Physiol Biochem 2018;49(6):2111–2123. doi:10.1159/000493815, PMID:30273928.

[67] Chen G, Zhang B, Xu H, Sun Y, Shi Y, Luo Y, *et al*. Suppression of Sirt1 sensitizes lung cancer cells to WEE1 inhibitor MK-1775-induced DNA damage and apoptosis. Oncogene 2017;36(50):6863–6872. doi:10.1038/onc.2017.297, PMID:28869605.

[68] Uhl M, Csernok A, Aydin S, Kreienberg R, Wiesmüller L, Gatz SA. Role of SIRT1 in homologous recombination. DNA Repair (Amst) 2010;9(4):383–393. doi:10.1016/j.dnarep.2009.12.020, PMID:20097625.

[69] Lin YH, Yuan J, Pei H, Liu T, Ann DK, Lou Z. KAP1 Deacetylation by SIRT1 Promotes Non-Homologous End-Joining Repair. PLoS One 2015;10(4):e0123935. doi:10.1371/journal.pone.0123935, PMID:259057 08.

[70] Dobbin MM, Madabhushi R, Pan L, Chen Y, Kim D, Gao J, *et al*. SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. Nat Neurosci 2013;16(8):1008–1015. doi:10.1038/nn.3460, PMID:23852118.

[71] Sundaresan NR, Samant SA, Pillai VB, Rajamohan SB, Gupta MP. SIRT3 is a stress-responsive deacetylase in cardiomyocytes that protects cells from stress-mediated cell death by deacetylation of Ku70. Mol Cell Biol 2008;28(20):6384–6401. doi:10.1128/MCB.00426-08, PMID:18710944.

[72] Chen Y, Zhang H, Xu Z, Tang H, Geng A, Cai B, *et al*. A PARP1-BRG1-SIRT1 axis promotes HR repair by reducing nucleosome density at DNA damage sites. Nucleic Acids Res 2019;47(16):8563–8580. doi:10.1093/nar/

gkz592, PMID:31291457.

[73] Wang P, Song X, Cao D, Cui K, Wang J, Utpatel K, *et al*. Oncogene-dependent function of BRG1 in hepatocarcinogenesis. Cell Death Dis 2020;11(2):91. doi:10.1038/s41419-020-2289-3, PMID:32019910.

[74] Chen Z, Gao Y, Yao L, Liu Y, Huang L, Yan Z, *et al*. LncFZD6 initiates Wnt/β-catenin and liver TIC self-renewal through BRG1-mediated FZD6 transcriptional activation. Oncogene 2018;37(23):3098–3112. doi:10.1038/s41388-018-0203-6, PMID:29535420.

[75] Jin H, Lee B, Luo Y, Choi Y, Choi EH, Jin H, *et al*. FOXL2 directs DNA double-strand break repair pathways by differentially interacting with Ku. Nat Commun 2020;11(1):2010. doi:10.1038/s41467-020-15748-1, PMID:32332759.

[76] Nguyen P, Shukla S, Liu R, Abbineni G, Smart DK. Sirt2 Regulates Radiation-Induced Injury. Radiat Res 2019;191(5):398–412. doi:10.1667/RR15282.1, PMID:30835165.

[77] Minten EV, Kapoor-Vazirani P, Li C, Zhang H, Balakrishnan K, Yu DS. SIRT2 promotes BRCA1-BARD1 heterodimerization through deacetylation. Cell Rep 2021;34(13):108921. doi:10.1016/j.celrep.2021.108921, PMID:33789098.

[78] Yasuda T, Takizawa K, Ui A, Hama M, Kagawa W, Sugasawa K, *et al*. Human SIRT2 and SIRT3 deacetylases function in DNA homologous recombinational repair. Genes Cells 2021;26(5):328–335. doi:10.1111/gtc.12842, PMID:33624391.

[79] Yasuda T, Kagawa W, Ogi T, Kato TA, Suzuki T, Dohmae N, *et al*. Novel function of HATs and HDACs in homologous recombination through acetylation of human RAD52 at double-strand break sites. PLoS Genet 2018; 14(3):e1007277. doi:10.1371/journal.pgen.1007277, PMID:29590107.

[80] Sengupta A, Haldar D. Human sirtuin 3 (SIRT3) deacetylates histone H3 lysine 56 to promote nonhomologous end joining repair. DNA Repair (Amst) 2018;61:1–16. doi:10.1016/j.dnarep.2017.11.003, PMID:29136592.

[81] Chen Y, Chen J, Sun X, Yu J, Qian Z, Wu L, *et al*. The SIRT6 activator MDL-800 improves genomic stability and pluripotency of old murine-derived iPS cells. Aging Cell 2020;19(8):e13185. doi:10.1111/acel.13185, PMID:33089974.

[82] Onn L, Portillo M, Ilic S, Cleitman G, Stein D, Kaluski S, *et al*. SIRT6 is a DNA double-strand break sensor. Elife 2020;9:e51636. doi:10.7554/eLife.51636, PMID:31995034.

[83] Mao Z, Tian X, Van Meter M, Ke Z, Gorbunova V, Seluanov A. Sirtuin 6 (SIRT6) rescues the decline of homologous recombination repair during replicative senescence. Proc Natl Acad Sci U S A 2012;109(29):11800–11805. doi:10.1073/pnas.1200583109, PMID:22753495.

[84] Hou T, Cao Z, Zhang J, Tang M, Tian Y, Li Y, *et al*. SIRT6 coordinates with CHD4 to promote chromatin relaxation and DNA repair. Nucleic Acids Res 2020;48(6):2982–3000. doi:10.1093/nar/gkaa006, PMID:31970415.

[85] Toiber D, Erdel F, Bouazoune K, Silberman DM, Zhong L, Mulligan P, *et al*. SIRT6 recruits SNF2H to DNA break sites, preventing genomic instability through chromatin remodeling. Mol Cell 2013;51(4):454–468. doi:10.1016/j.molcel.2013.06.018, PMID:23911928.

[86] Rezazadeh S, Yang D, Biashad SA, Firsanov D, Takasugi M, Gilbert M, *et al*. SIRT6 mono-ADP ribosylates KDM2A to locally increase H3K36me2 at DNA damage sites to inhibit transcription and promote repair. Aging (Albany NY) 2020;12(12):11165–11184. doi:10.18632/aging.103567, PMID:325 84788.

[87] McCord RA, Michishita E, Hong T, Berber E, Boxer LD, Kusumoto R, *et al*. SIRT6 stabilizes DNA-dependent protein kinase at chromatin for DNA double-strand break repair. Aging (Albany NY) 2009;1(1):109–121. doi: 10.18632/aging.100011, PMID:20157594.

[88] Chen W, Liu N, Zhang H, Zhang H, Qiao J, Jia W, *et al*. Sirt6 Promotes DNA End Joining in iPSCs Derived from Old Mice. Cell Rep 2017;18(12):2880–2892. doi:10.1016/j.celrep.2017.02.082, PMID:28329681.

[89] Ke Z, Firsanov D, Spencer B, Seluanov A, Gorbunova V. Short-term calorie restriction enhances DNA repair by non-homologous end joining in mice. NPJ Aging Mech Dis 2020;6:9. doi:10.1038/s41514-020-00047-2, PMID:32864160.

[90] Saksouk N, Hajdari S, Perez Y, Pratlong M, Barrachina C, Graber C, *et al*. The mouse HP1 proteins are essential for preventing liver tumorigenesis. Oncogene 2020;39(13):2676–2691. doi:10.1038/s41388-020-1177-8, PMID: 32020053.

[91] Nio K, Yamashita T, Okada H, Kondo M, Hayashi T, Hara Y, *et al*. Defeating Ep-CAM(+) liver cancer stem cells by targeting chromatin remodeling enzyme CHD4 in human hepatocellular carcinoma. J Hepatol 2015;63(5):1164–1172. doi:10.1016/j.jhep.2015.06.009, PMID:26095183.

[92] Shao S, Cao H, Wang Z, Zhou D, Wu C, Wang S, *et al*. CHD4/NuRD complex regulates complement gene expression and correlates with CD8 T cell infiltration in human hepatocellular carcinoma. Clin Epigenetics 2020;12(1):31. doi:10.1186/s13148-020-00827-3, PMID:32070428.

[93] Lin Q, Wu Z, Yue X, Yu X, Wang Z, Song X, *et al*. ZHX2 restricts hepatocellular carcinoma by suppressing stem cell-like traits through KDM2A-mediated H3K36 demethylation. EBioMedicine 2020;53:102676. doi:10.1016/j.ebiom.2020.102676, PMID:32114388.

[94] Vazquez BN, Thackray JK, Simonet NG, Kane-Goldsmith N, Martinez-Redondo P, Nguyen T, *et al*. SIRT7 promotes genome integrity and modulates non-homologous end joining DNA repair. EMBO J 2016;35(14):1488–1503. doi:10.15252/embj.201593499, PMID:27225932.

[95] Ianni A, Hoelper S, Krueger M, Braun T, Bober E. Sirt7 stabilizes rDNA heterochromatin through recruitment of DNMT1 and Sirt1. Biochem Biophys Res Commun 2017;492(3):434–440. doi:10.1016/j.bbrc.2017.08.081, PMID:28842251.

[96] Chen X, Li WF, Wu X, Zhang HC, Chen L, Zhang PY, *et al*. Dicer regulates non-homologous end joining and is associated with chemosensitivity in colon cancer patients. Carcinogenesis 2017;38(9):873–882. doi:10.1093/

carcin/bgx059, PMID:28911000.

[97] Quint K, Agaimy A, Di Fazio P, Montalbano R, Steindorf C, Jung R, *et al*. Clinical significance of histone deacetylases 1, 2, 3, and 7: HDAC2 is an independent predictor of survival in HCC. Virchows Arch 2011;459(2):129–139. doi:10.1007/s00428-011-1103-0, PMID:21713366.

[98] Amano H, Chaudhury A, Rodriguez-Aguayo C, Lu L, Akhanov V, Catic A, *et al*. Telomere Dysfunction Induces Sirtuin Repression that Drives Telomere-Dependent Disease. Cell Metab 2019;29(6):1274–1290.e9. doi:10.1016/j.cmet.2019.03.001, PMID:30930169.

[99] Pfluger PT, Herranz D, Velasco-Miguel S, Serrano M, Tschöp MH. Sirt1 protects against high-fat diet-induced metabolic damage. Proc Natl Acad Sci U S A 2008;105(28):9793–9798. doi:10.1073/pnas.0802917105, PMID:18599449.

[100] You M, Jogasuria A, Taylor C, Wu J. Sirtuin 1 signaling and alcoholic fatty liver disease. Hepatobiliary Surg Nutr 2015;4(2):88–100. doi:10.3978/j.issn.2304-3881.2014.12.06, PMID:26005675.

[101] Pantazi E, Zaouali MA, Bejaoui M, Serafin A, Folch-Puy E, Petegnief V, *et al*. Silent information regulator 1 protects the liver against ischemia-reperfusion injury: implications in steatotic liver ischemic preconditioning. Transpl Int 2014;27(5):493–503. doi:10.1111/tri.12276, PMID:24472096.

[102] Gavande NS, VanderVere-Carozza PS, Hinshaw HD, Jalal SI, Sears CR, Pawelczak KS, *et al*. DNA repair targeted therapy: The past or future of cancer treatment? Pharmacol Ther 2016;160:65–83. doi:10.1016/j.pharmthera.2016.02.003, PMID:26896565.

[103] Javle M, Curtin NJ. The role of PARP in DNA repair and its therapeutic exploitation. Br J Cancer 2011;105(8):1114–1122. doi:10.1038/bjc.2011.382, PMID:21989215.

[104] Li G, Tian Y, Zhu WG. The Roles of Histone Deacetylases and Their Inhibitors in Cancer Therapy. Front Cell Dev Biol 2020;8:576946. doi:10.3389/fcell.2020.576946, PMID:33117804.

[105] Tsilimigras DI, Ntanasis-Stathopoulos I, Moris D, Spartalis E, Pawlik TM. Histone deacetylase inhibitors in hepatocellular carcinoma: A therapeutic perspective. Surg Oncol 2018;27(4):611–618. doi:10.1016/j.suronc.2018.07.015, PMID:30449480.

[106] Valdez BC, Li Y, Murray D, Liu Y, Nieto Y, Champlin RE, *et al*. Combination of a hypomethylating agent and inhibitors of PARP and HDAC traps PARP1 and DNMT1 to chromatin, acetylates DNA repair proteins, downregulates NuRD and induces apoptosis in human leukemia and lymphoma cells. Oncotarget 2018;9(3):3908–3921. doi:10.18632/oncotarget.23386, PMID:29423093.

[107] Purrucker JC, Fricke A, Ong MF, Rübe C, Rübe CE, Mahlknecht U. HDAC inhibition radiosensitizes human normal tissue cells and reduces DNA Double-Strand Break repair capacity. Oncol Rep 2010;23(1):263–269. PMID:19956891.

[108] Kaiser GS, Germann SM, Westergaard T, Lisby M. Phenylbutyrate inhibits homologous recombination induced by camptothecin and methyl methanesulfonate. Mutat Res 2011;713(1-2):64–75. doi:10.1016/j.mrfmmm.2011.05.016, PMID:21658395.

[109] Chung YL, Pui NN. Dynamics of wound healing signaling as a potential therapeutic target for radiation-induced tissue damage. Wound Repair Regen 2015;23(2):278–286. doi:10.1111/wrr.12265, PMID:25682986.

[110] Maiso P, Colado E, Ocio EM, Garayoa M, Martín J, Atadja P, *et al*. The synergy of panobinostat plus doxorubicin in acute myeloid leukemia suggests a role for HDAC inhibitors in the control of DNA repair. Leukemia 2009;23(12):2265–2274. doi:10.1038/leu.2009.182, PMID:19812608.

[111] Wilson AJ, Sarfo-Kantanka K, Barrack T, Steck A, Saskowski J, Crispens MA, *et al*. Panobinostat sensitizes cyclin E high, homologous recombination-proficient ovarian cancer to olaparib. Gynecol Oncol 2016;143(1):143–151. doi:10.1016/j.ygyno.2016.07.088, PMID:27444036.

[112] Xiao Y, Lin FT, Lin WC. ACTL6A promotes repair of cisplatin-induced DNA damage, a new mechanism of platinum resistance in cancer. Proc Natl Acad Sci U S A 2021;118(3):e2015808118. doi:10.1073/pnas.2015808118, PMID:33408251.

[113] Munshi A, Tanaka T, Hobbs ML, Tucker SL, Richon VM, Meyn RE. Vorinostat, a histone deacetylase inhibitor, enhances the response of human tumor cells to ionizing radiation through prolongation of gamma-H2AX foci. Mol Cancer Ther 2006;5(8):1967–1974. doi:10.1158/1535-7163.MCT-06-0022, PMID:16928817.

[114] Attia SM, Al-Khalifa MK, Al-Hamamah MA, Alotaibi MR, Attia MSM, Ahmad SF, *et al*. Vorinostat is genotoxic and epigenotoxic in the mouse bone marrow cells at the human equivalent doses. Toxicology 2020;441:152507. doi:10.1016/j.tox.2020.152507, PMID:32512035.

[115] Lee JH, Choy ML, Ngo L, Foster SS, Marks PA. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. Proc Natl Acad Sci U S A 2010;107(33):14639–14644. doi:10.1073/pnas.1008522107, PMID:20679231.

[116] Yang H, Lan P, Hou Z, Guan Y, Zhang J, Xu W, *et al*. Histone deacetylase inhibitor SAHA epigenetically regulates miR-17-92 cluster and MCM7 to upregulate MICA expression in hepatoma. Br J Cancer 2015;112(1):112–121. doi:10.1038/bjc.2014.547, PMID:25393367.

[117] Srinivas C, Swathi V, Priyanka C, Anjana Devi T, Subba Reddy BV, Janaki Ramaiah M, *et al*. Novel SAHA analogues inhibit HDACs, induce apoptosis and modulate the expression of microRNAs in hepatocellular carcinoma. Apoptosis 2016;21(11):1249–1264. doi:10.1007/s10495-016-1278-6, PMID:27502208.

[118] Gordon SW, McGuire WP 3rd, Shafer DA, Sterling RK, Lee HM, Matherly SC, *et al*. Phase I Study of Sorafenib and Vorinostat in Advanced Hepatocellular Carcinoma. Am J Clin Oncol 2019;42(8):649–654. doi:10.1097/COC.0000000000000567, PMID:31305287.

[119] Sun WJ, Huang H, He B, Hu DH, Li PH, Yu YJ, *et al*. Romidepsin induces G2/M phase arrest via Erk/cdc25C/cdc2/cyclinB pathway and apoptosis induction through JNK/c-Jun/caspase3 pathway in hepatocellular carcinoma cells. Biochem Pharmacol 2017;127:90–100. doi:10.1016/j.bcp.2016.12.008, PMID:28012958.

[120] Pathil A, Armeanu S, Venturelli S, Mascagni P, Weiss TS, Gregor M, *et al*. HDAC inhibitor treatment of hepatoma cells induces both TRAIL-independent apoptosis and restoration of sensitivity to TRAIL. Hepatology 2006;43(3):425–434. doi:10.1002/hep.21054, PMID:16583461.

[121] Sun G, Mackey LV, Coy DH, Yu CY, Sun L. The Histone Deacetylase Inhibitor Vaproic Acid Induces Cell Growth Arrest in Hepatocellular Carcinoma Cells via Suppressing Notch Signaling. J Cancer 2015;6(10):996–1004. doi:10.7150/jca.12135, PMID:26366213.

[122] Armeanu S, Bitzer M, Lauer UM, Venturelli S, Pathil A, Krusch M, *et al*. Natural killer cell-mediated lysis of hepatoma cells via specific induction of NKG2D ligands by the histone deacetylase inhibitor sodium valproate. Cancer Res 2005;65(14):6321–6329. doi:10.1158/0008-5472.CAN-04-4252, PMID:16024634.

[123] Montalbano R, Waldegger P, Quint K, Jabari S, Neureiter D, Illig R, *et al*. Endoplasmic reticulum stress plays a pivotal role in cell death mediated by the pan-deacetylase inhibitor panobinostat in human hepatocellular cancer cells. Transl Oncol 2013;6(2):143–157. doi:10.1593/tlo.12271, PMID:23544167.

[124] Song X, Wang J, Zheng T, Song R, Liang Y, Bhatta N, *et al*. LBH589 Inhibits proliferation and metastasis of hepatocellular carcinoma via inhibition of gankyrin/STAT3/Akt pathway. Mol Cancer 2013;12(1):114. doi:10.1186/1476-4598-12-114, PMID:24093956.

[125] Bitzer M, Horger M, Giannini EG, Ganten TM, Wörns MA, Siveke JT, *et al*. Resminostat plus sorafenib as second-line therapy of advanced hepatocellular carcinoma - The SHELTER study. J Hepatol 2016;65(2):280–288. doi:10.1016/j.jhep.2016.02.043, PMID:26952006.