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

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Received: 14 February 2022 | Revised: 9 July 2022 | Accepted: 20 July 2022 | Published: 13 September 2022

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.

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.

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. 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. 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. Taken together, these results emphasize multiple functions of HDAC 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. 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 damage repair.
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, chemotherapy drugs, and intrinsic agents, such as oxygen radicals and metabolites. 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 G2 phases, is a highly conservative and faultless mechanism. 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/PPR1, PARP1 and NuRD, ATM, ATR, CHK1/2, TIP60, and CtIP.

DNA2, a sensor for DNA damage, controls MRN-directed resection. Phosphorylated RPA loading at ssDNA as a bridge is replaced by recombinaise 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. The filament, in order to repair the lesion, may either undergo the synthesis-dependent strand annealing (SDSA) pathway, engagement with 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.

NHEJ

A rapid but not sufficiently accurate mechanism compared with HR, NHEJ mainly occurs in G1 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). DNA-PKcs and Ku together constitute the Ku/DNA-PKcs complex as DNA-dependent protein kinase (DNA-PK). DNA-PKcs undergoes autophosphorylation and then recruits and phosphorylates Artemis. 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 dissipative DNA end. After that, DNA polymerases (pol), including pol λ, pol δ and terminal deoxynucleotidyl transferase (TdT), are involved in ligation. 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.

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. Poly(ADP-ribose) polymerase 1 (PARP1) is involved in sensing DNA damage and binding the end of the DNA. The MRN complex, which is phosphorylated by CtIP and initiates alt-EJ, can be inhibited by Ku competing combination with DSBs. Alt-EJ can start only if the content of Ku hovers at a relatively low level. The MRN generates 15- to 100-nucleotide 3′ overlaps through its endonuclease function, exhibiting the microhomology of DSBs, where the DNA pol 8 extends the DNA ends, utilizing the opposite DNA sequence as a replication template. The stable annealing partner is ultimately sealed by DNA ligase I or DNA ligase III.

SSA

SSA, as a backup to HR, is prone to induce mutations along with severe deletions and translocations. 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. MRN and CtIP are involved in creating the 3 DNA tails, and then EXO1, BLM, and DNA2 extend the tails. Multiple copies of RPA combine with the prolonged DNA end for stability and protection, lessening the formation of secondary structures. After that, RAD52 substitutes for RPA for the DNA strand invasion and annealing. Furthermore, the redundant unannealed flaps are removed by the ERCC1/XPF nuclease, and possible
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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). 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. p53-binding protein 1 (53BP1) binds to DNA ends and form irradiation-induced foci, limiting the length of resection and prompting NHEJ. The function of the Shieldin complex is similar to that of 53BP1, blocking DNA end resection and inducing NHEJ. 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. Moreover, BRCA2 and RAD51 can overcome the resistance of 53BP1 and the Shieldin complex toward DNA end resection and recover the HR pathway. 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. 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. 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.

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,
The accumulation of the signaling cascade initiated by DNA γH2AX. This phosphorylation event serves as an anchor for damage, H2AX is phosphorylated at serine 139 to generate and 5 can be found.36 HDACs not only epigenetically modify found in the cytoplasm. In the mitochondria, SIRTs 3, 4, silent information regulation-2 (Sir2). SIRTs 1, 2, 6 and 7 pounds such as hydroxamic acids. Sirtuins (SIRTs) are de-
dependent enzymes harboring a catalytic pocket with a Zn 2+ cation at its base that can be inhibited by Zn2+-chelating com-
pounds such as hydroxamic acids. Sirtuins (SIRTs) are de-
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.30 HDACs not only epigenetically modify histone acetylation but also deacetylate various crucial fac-
tors 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 de-
creases DNA repair and causes DNA damage that is sig-
ificantly 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

Class I HDACs and DNA repair

HDAC1-3 are all highly expressed in HCC, correlating with tumor dedifferentiation and proliferative activity.57 HDAC1 and HDAC2 participate in the DDR through their location in DSB foci and coupled 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.59 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 recruit-
to the promoter of miR-182 to diminish its expression, thus promoting HR.44 In HCC tissue, miR-182 suppresses forhead 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 regu-
lators, 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 initi-
ate HR and NHEJ.46 Meanwhile, inactivation of HDAC3 also leads to the accreted acetylation of a series of sites on H4, such as H4K5/12, 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.53 BP1 and yH2AX 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 my-
eloid 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 inhibi-
tion of HDAC4 reduces cell viability, the activation of AKT

![Fig. 3. Overview of non-homologous end joining. The Ku70–Ku80 hetero-
dimer 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 cata-
lytic subunit; DSB, double strand break; XRCC4, X-ray repair cross-comple-
menting 4; NHEJ, nonhomologous end joining; XLF, XRCC4-like factor.]

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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 Ⅲ 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, γ-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               |              |                |                |                   |                      | 57 |
| **Class IIB**       |              |                |                |                   |                      |           |
| HDAC6*              | SMAR1, Ku70, BAX |                |                | NHE↑ |                      | 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.
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. 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.

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. 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.

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. 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. In high-grade serous ovarian carcinomas, HDAC6 removes acetylation from H4K12 and H4K16, inducing HR deficiency, which increases the sensitivity to chemotherapy. In contrast, HDAC6 activates its downstream factor, Sp1, to upregulate RAD51, CHEK1, EXO1, RAD54L, and GEN1, promoting HR repair in glioblastoma cells.

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.

**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 highly selective. Nonmitochondrial SIRTs are affected by PGC-1α and PGC-1β, whereas mitochondrial SIRTs are regulated by miR-34a, 26a, and 145.

**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. In liver tissue with ischemic injury, SIRT1 expression and activity are upregulated to compensate for injury. This function can be abrogated by SIRT1 knockdown. In HCC tissues, SIRT1 mainly acts as an onco- gene that mediates tumorigenesis and chemoresistance, promoting HCC proliferation and indicating poor prognosis in patients with liver cancer.

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. SIRT1 is the most important deacetylase of Ku70, and inhibition of SIRT1 enhances Ku70 acetylation, thereby directly obstructing the NHEJ repair pathway. SIRT1 can also remove acetylation from Kap1, thus stabilizing the interaction between Kap1 and 53BP1 to respond to DSBs and promoting NHEJ. In addition, SIRT1 deacetylates HDAC1 and mediates the NHEJ repair function. Finally, SIRT1 is a crucial mediator of the SIRT1-FOXO2-XRCC5/6 axis. FOXO2, 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.

Inactivated SIRT1 causes a reduction in RAD51, indicating that the HR repair pathway is also regulated by SIR1.
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, deacetylation on NBS1 can be substituted with phosphorylation by SIRT1, as well as ATM, to promote HR.

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.

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. SIRT1 also deacetylates nibrin and WRN helicase to promote MRN complex generation for HR initiation.

**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 I and XRCC4, therefore improving HR and NHEJ efficiency.

SIRT2 deacetylases conserve lysine residues of BARD1 to enable BRCA1 binding, thus catalyzing BRCA1-BARD1 heterodimerization to maintain their mutual stability, promoting HR and prohibiting tumorigenesis. 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.

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.

**SIRT6**

SIRT6 acts as a longevity gene that wields various functions to retain cell viability in aging cells, such as maintaining genome integrity. In aging cells, SIRT6 and NHEJ are downregulated, while short-term calorie restriction is associated with increased levels of DNA-PK and SIRT6 to maintain cell viability in aging cells, such as maintaining genome integrity. In aging cells, SIRT6 and NHEJ are downregulated, while short-term calorie restriction is associated with increased levels of DNA-PK and SIRT6 to maintain cell viability in aging cells, such as maintaining genome integrity.

SIRT6 possesses NAD⁺-dependent protein deacetylase activity and mono (ADP-ribosyl) transferase activity, acting as a cross point between DNA repair and transcription. Depletion of SIRT6 reduces the expression of several DNA repair proteins, including Rad51, Artemis, DNA ligase I and XRCC4, therefore improving HR and NHEJ efficiency.

SIRT6 can interact with Ku80 to enable Ku80 to combine with DNA-PKcs, enhancing DNA-PKcs phosphorylation. SIRT6 can be recruited by PARP1 to DSBS sites to deacetylate H3K18ac for 53BP1 loading to start NHEJ. 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.

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.

**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. Meanwhile, inhibition of DNA 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. PARP1 is significantly upregulated in embryonic stem cells and re-
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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 2.106-112 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 cytolyis 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-

### 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/cdc2/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 | P21WAF1/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 radiosensitivity 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 |

Residual 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.

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The efficacy of SAHA in the treatment of HCC deserves further study. Although further study was terminated due to the high incidence of toxicities in patients, the majority of them have 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 HDACi play a positive role in improving patient survival and symptom control, in most cases, HCC cells develop drug resistance to HDACi, 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.\textsuperscript{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 HDACi might explain the efficacy of HDACi 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 HDACi 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.

Acknowledgments

We would like to express our thanks to Ms. Mari Ekimyan Salvo for her guidance and professional advice on writing the review.

Funding

This work was supported by grants from the Science and Technology Research Program of Chongqing Education Commission (no. KJQN202100424) and the Natural Science Foundation Project of Chongqing (no. cstc2018jcyjAX0825).

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, QJ).

Limitations of HDACi on HCC treatment

Although many HDACis have been developed, the vast majority of them have 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 HDACi play a positive role in improving patient survival and symptom control, in most cases, HCC cells develop drug resistance to HDACi, resulting in malignant phenotype regeneration and maintenance.
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