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REVIEW ARTICLE

Targeting chromatin to improve radiation response

M M OLCINA, PhD, S O'DELL, BSc and E M HAMMOND, PhD

CR-UK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, UK

Address correspondence to: Dr Ester M Hammond

E-mail: ester.hammond@oncology.ox.ac.uk

ABSTRACT

Chromatin, the structure formed by the wrapping of approximately 146 base pairs of DNA around an octamer of histones, has a profound impact on numerous DNA-based processes. Chromatin modifications and chromatin remodellers have recently been implicated in important aspects of the DNA damage response including facilitating the initial sensing of the damage as well as subsequent recruitment of repair factors. Radiation is an effective cancer therapy for a large number of tumours, and there is considerable interest in finding approaches that might further increase the efficacy of radiotherapy. The use of radiation leads to the generation of DNA damage and, therefore, agents that can affect the sensing and repair of DNA damage may have an impact on overall radiation efficacy. The chromatin modifications as well as chromatin modifiers that have been associated with the DNA damage response will be summarized in this review. An emphasis will be placed on those processes that can be pharmacologically manipulated with currently available inhibitors. The rationale for the use of these inhibitors in combination with radiation will also be described.

Radiotherapy is an effective therapy for around 50% of patients.¹ Radiation leads to the direct damage of DNA as well as the formation of radicals, which result in the generation of lesions such as single-strand break (SSB), double-strand break (DSB) and base changes. These DNA lesions result in the activation of the DNA damage response (DDR), which is a complex signalling cascade.² This response is induced in an attempt to halt the cell cycle in order to repair the damage or induce apoptosis or senescence if the damage is too severe (shown schematically in Figure 1 and reviewed in Sulli et al³). The DDR is initiated by signalling via apical kinases including ATR, ATM and DNA-PK.⁴ Targeting the DDR has been proposed as a method of enhancing the effects of radiation. Inhibitors of critical components to the DDR such as CHK1, ATR or ATM have shown promise in a number of cancer cell types *in vitro* as well as *in vivo*.^{5–9}

DNA damage does not occur in isolation but rather within the context of the surrounding chromatin. Chromatin refers to the structure formed by the wrapping of approximately 146 base pairs of DNA around an octamer of histones consisting of H2A, H2B, H3 and H4. It is now becoming apparent that chromatin changes can have a profound impact on the DDR.^{3,10,11} This can be by physically protecting the DNA from damage, affecting the spread of DDR signalling or by regulating the mechanism of response to DNA damage within specific chromatin

environments.³ Evidence suggests, for example, that following radiation, DNA that is lacking histones or other chromatin components sustains a greater degree of damage than does DNA associated with high chromatin compaction.^{12,13}

In this review, chromatin changes that have been linked to changes in DDR signalling and radiation sensitivity will be described. Emphasis will be placed on those chromatin modifications that can be targeted through the use of inhibitors and how the use of such compounds could be combined with radiation to improve radiotherapy outcomes.

CHROMATIN MODULATION

The term “epigenetics” was originally coined by Conrad Waddington to describe changes in the cellular phenotype that, while heritable, were observed to be independent of alterations to the DNA sequence. Today, modifications involving DNA, histones, histone variants as well as chromatin remodellers are known to be both dynamic and highly regulated.^{14–17} Post-translational modification of histones has been widely characterized with histone acetylation and methylation typically receiving most attention. The N-acetylation of histones (especially on lysine residues), catalysed by histone acetyltransferases (HATs), has been characterized and is involved in transcriptional regulation, chromatin structure control and DNA repair. The addition of an acetyl group neutralizes the positive charge of lysine and is thought to weaken the electrostatic interaction

Figure 1. Schematic representation of the DNA damage response (DDR). A simplified representation of the DDR is shown to illustrate the signalling cascade that is triggered in response to DNA damage. Examples of the molecular components are given. As a result of the activation of the DDR, cells can attempt to repair the DNA damage. If the damage is unrepairable, the cell can be directed to apoptosis or senescence to ensure mutations are not transmitted to daughter cells.

present between charged DNA and histones. Therefore, histone acetylation is associated with more open chromatin structures and active transcription. Furthermore, lysine acetylation allows for binding of proteins with bromodomains and plant homeodomain fingers capable of recognizing this covalent modification.¹⁸ Not surprisingly, the removal or “erasing” of these marks by histone deacetylases (HDACs) leads to a condensation of chromatin and correlates with transcriptional repression/silencing.¹⁹

Histone methylation can occur on basic amino acid residues such as lysine and arginine. Methylation is carried out by histone methyltransferases (HMTs) and is dependent on the donation of a methyl group from S-adenosyl methionine (SAM).²⁰ Lysine residues may be mono-, di- or tri-methylated by the lysine methyltransferases (KMTs), a group of enzymes that were originally categorized by the presence of either a suppressor of variation 3–9, enhancer of zeste trithorax (SET) or DOT1-like (DOT1L) domain.^{20,21} Protein arginine methyltransferases are responsible for the methylation of arginine residues and are grouped into two types dependent on the symmetry of their

methyl group addition.²⁰ Histone methylation does not alter the charge of the histone tail (unlike acetylation), but instead modifies the hydrophobicity and basicity of histones and therefore the affinity for other proteins, such as transcription factors.²² Histone methylation is highly contextual in its biological effects and may result in either activation or repression of transcription dependant on the location of the modification. For example, methylation of lysine residues has been correlated with activation of transcription (H3K4/36/79) as well as transcriptional silencing (H3K9, H4K20).²⁰ The complex code of histone methylation, in addition to the role of these enzymes within the context of multiprotein complexes, makes these enzymes more difficult to target pharmacologically. As a direct result, HMT inhibitors (HMTi) have not yet reached the clinic, although there is evidence for their roles in cancer, therefore ensuring continued investigation of their therapeutic potential.²³

As their name implies histone demethylases (HDMs) are responsible for histone demethylation. To date this group of enzymes consist of lysine-specific demethylases (KDM) and a single arginine-specific demethylase (RDM). The KDM include two families: those that contain the amine oxidase-like (AOL) domain and those with the Jumonji domain (JmjC).²³ Only two members of the AOL domain family are known, KDM1A (LSD1) and KDM1B (LSD2).²⁴ These enzymes specifically catalyse the removal of mono- and di-methylation from H3K4, but change specificity when in complex with different accessory proteins.²⁵ The Jumonji C (JmjC) family currently has approximately 30 members, that are divided into 7 groups. They have been found to demethylate mono-, di- and tri-methylated lysines.²⁰

CHROMATIN AND THE DNA DAMAGE RESPONSE

Any damage to DNA occurs within a particular chromatin context. In the case of a DSB for instance, initial sensing of the damage and activation of upstream kinases can be influenced and facilitated by chromatin modifiers. The interaction of the chromatin context with the DDR is extremely complex and only a subset of events (and references) are shown in Table 1 (for a more comprehensive recent review of the topic, see Sulli *et al*).³ Upstream/apical kinases such as ATM, ATR and DNA-PK can mediate chromatin changes themselves, perhaps the most widely characterized of these being H2AX phosphorylation (γ H2AX).^{38,39} Mediator proteins acting downstream of these apical kinases help create a positive-feedback loop to sustain the DDR signalling. Some of these mediators such as MDC1 recruit E3 ubiquitin ligases such as UBC13-RNF8 to allow H2AX ubiquitylation, an important event for the recruitment of factors that facilitate repair such as 53BP1 or BRCA1.^{40–43} HATs such as TIP60 can also relax chromatin at DSBs by mediating acetylation on H4 and H2A.^{44–46} TIP60-dependent acetylation of H2AX on Lys 5 is required for ubiquitylation of H2AX.²⁹ Furthermore, HDAC1 and HDAC2 localize to sites of damage leading to a reduction in acetylation on H3K56. Functionally, cells where HDAC1 and HDAC2 have been depleted display marked defects in non-homologous end joining (NHEJ) and may also have defective homologous recombination-mediated repair.³⁵

While H2A histone modifications are important for coordinating an appropriate DDR, histone methylation has also

Table 1. An overview of selected chromatin modifiers in DNA damage response (DDR) signalling and repair

Name	Type	Specificity	Role in repair/DDR	Reference
KDM2A/FBXL11/JHDM1A	JmjC HDM	H3K36me1 H3K4me3	Enhances NHEJ-mediated repair	Fnu et al ²⁶
KDM4B/JMJD2B/JHDM3B	JmjC HDM	H3K9me2/3	Increased KDM4B leads to less IR-induced H2AX and increased cell survival	Young et al ²⁷
DOT1L	HMT	H3K79me2	53BP1 recruitment	Huyen et al ²⁸
TIP60	HAT	Various including ATM, p53 and H2AK5ac	Lysine 5 acetylation of H2AX is necessary for ubiquitylation of H2AX ATM acetylation facilitates ATM autophosphorylation and activation	Ikura et al, ²⁹ Sun et al, ³⁰ Kaidi and Jackson ³¹
CHD3	ATP-ase chromatin remodeller	Various	Mediates heterochromatin formation preventing DSB expansion	Goodarzi et al ³²
CHD4	ATP-ase chromatin remodeller	Various	Promotes DSB repair possibly specifically during S/G ₂ phase	Chou et al, ³³ Larsen et al ³⁴
HDAC	HDAC	Various	Facilitates HR and NHEJ Facilitates ATM signalling	Miller et al ³⁵
SWI/SNF	ATP-ase chromatin remodeller	Various	Facilitates DSB repair	van Attikum et al ³⁶
CBP	HAT	H3K56ac	Checkpoint and chromatin assembly recovery	Vempati et al ³⁷

DSB, double strand break; HAT, histone acetyltransferase; HDAC, histone deacetylase; HDM, histone demethylase; HMT, histone methyltransferase; HR, homologous recombination; IR, ionizing radiation; JmjC, Jumonji domain; NHEJ, non-homologous end joining.

been shown to play an important role. H3K9me3 appears important for TIP60-dependent ATM acetylation, an event that facilitates ATM activation.^{30,47} Furthermore, DOT1L histone H3K79 methyltransferase-dependent dimethylation may facilitate 53BP1 recruitment following DNA damage through its tandem tudor domain.²⁸ DOT1L depletion may result in reductions in H3K79me2 and 53BP1 foci formation. It is interesting to note, however, that H3K79me2 levels are not altered following radiation.²⁸ More recently, it has become apparent that 53BP1 appears to have a greater affinity for H4K20me1 than for H3K79me2 and silencing of the SET domain-containing KMT 8 (SETD8/PRSET7), the methyltransferase catalysing H4K20me1, impairs 53BP1 recruitment. In addition, multiple myeloma SET domain-containing protein (MMSET/WHSC1), the methyltransferase responsible for H4K20 di- and trimethylation, is also required for 53BP1 localization. Both MMSET and PRSET7 are recruited to sites of DNA damage and consequently levels of H4K20me1/2/3 are induced around a DNA break site.⁴⁸ Recruitment of PRSET7 has recently been shown to be dependent on Ku70 and appears important for appropriate NHEJ.⁴⁹ Recruitment of PRSET7 also facilitates dimethylation at H4K20 by the Suv4-20 methyltransferase an event that is again required for 53BP1 nucleation.⁴⁹ The importance of H4K20 methylation in 53BP1 recruitment has been demonstrated further by the finding that the tandem tudor domain of the HDM JMJD2A can recognize H4K20me2, thereby preventing 53BP1 recruitment in the absence of damage. RNF8 and RNF168 target this demethylase for ubiquitylation and proteasomal degradation following ionizing radiation (IR) to allow 53BP1 foci formation.⁵⁰ Recently, H4K16 acetylation has been

suggested to impair 53BP1 binding to H4K20me2, with histone deacetylation occurring in response to DNA damage facilitating the formation of 53BP1 foci.⁵¹

Several other HDMs have also recently been demonstrated to play a role in DNA damage signalling. H3K4me3 levels have been shown to be reduced at the sites of DSBs.⁵² Interestingly, H3K4me3 demethylase LSD1 is recruited to DSBs, a process facilitated by interaction between LSD1 and RNF168. shRNA-mediated knockdown of LSD1 results in reduced H2A/H2AX ubiquitylation and reduced 53BP1 and BRCA1 recruitment following DNA damage. In addition, LSD1 knockdown confers mild sensitivity to IR.⁵² Notably, the H3K9me2/3 demethylase KDM4B is also recruited to DSB sites, and KDM4B overexpression results in reduced γ H2AX and increased cell survival following IR.²⁷

Similarly, hampering of DDR signalling has been shown to occur following the overexpression of bromodomain and extra terminal family member BRD4, specifically, BRD4 isoform B.⁵³ This isoform has recently been implicated in maintaining chromatin compaction around DSBs by recruiting the condensing II chromatin remodelling complex to acetylated histones, and thereby preventing efficient γ H2AX signalling following IR. Inhibition of BRD4 using JQ1 (a BRD4 inhibitor) results in decreased sensitivity to IR presumably through rapid checkpoint recovery following the increased chromatin relaxation achieved by decreased BRD4 isoform B activity. It is important to note, however, that inhibiting chromatin-modifying factors such as BRD4 might have differential effects depending on the cell type.⁵³

INHIBITING CHROMATIN CHANGES AND RADIOSENSITIVITY

Of the chromatin modifications, DNA methylation, in addition to histone methylation and acetylation, is generally the most widely characterized and has therefore been the major focus of novel therapeutic strategies in this area. Despite current questions and lingering gaps in knowledge, inhibitors of the enzymes involved in these modifications have and are being developed, and some show promise for cancer therapy.⁵⁴

DNA METHYLTRANSFERASE INHIBITORS

Some DNA methyltransferase inhibitors (DNMTi) have demonstrated clinical efficacy in the treatment of certain haematological malignancies.⁵⁵ Their use as single agents, however, has raised some concerns owing to mild efficacy and the profile of associated side effects. The use of lower doses of some of these inhibitors in combination with chemotherapy or radiotherapy has been proposed as a potentially useful means of using such inhibitors.⁵⁶ A number of mechanisms have been suggested as a rationale for the efficacy of these agents in combination with radiation (Table 2).⁵⁶ For example, the DNMTi that are nucleoside analogues, such as 5-Aza-cytidine (azacitidine), 5-aza-2'-deoxycytidine (decitabine) and zebularine, can create an irreversible DNA adduct (where the DNMT is bound to DNA and is therefore inhibited).^{60,61} When this occurs in close proximity to radiation-induced damage, such as a SSB, the break is more difficult to repair (Figure 2).⁶² Repair of radiation-induced DNA damage may also be reduced following treatment with nucleoside analogues, since these can hinder the progress of DNA synthesis.⁶³ Furthermore, it has been proposed that DNMTi could synchronize cells in the more radiosensitive phases of the cell cycle such as G₂/M. Moreover, since DNA methylation can lead to gene silencing, the use of a DNMTi can result in the reactivation of certain genes. Induction of apoptosis has been proposed to accompany DNMTi treatment potentially owing to the re-expression of specific genes such as RASSF1 or DAPK1 or reactivation of mediators of apoptosis.⁵⁶ Together, these effects suggest that some DNMTi could be used in a beneficial manner together with radiation.

HISTONE ACETYLTRANSFERASE INHIBITORS

Loss of TIP60 inhibits the effective repair of DNA damage and sensitizes cells to radiation, suggesting that TIP60 inhibitors could be therapeutically effective in combination with radiation.³⁰ Several "global" HAT inhibitors have been demonstrated to possess the ability to radiosensitize cancer cells, including

anacardic acid and curcumin. Despite permeability restrictions *in vitro*, anacardic acid has been found to block the TIP60-dependent activation of the ATM and DNA-PKcs protein kinases by DNA damage *in vivo*, therefore sensitizing human tumour cells to the cytotoxic effects of ionizing radiation.⁶⁴

HISTONE DEACETYLASE INHIBITORS

There has been considerable effort in investigating the benefits of HDAC inhibition in the context of cancer treatment. Two of the zinc-dependent HDAC inhibitors (HDACi) (vorinostat and romidespin) have been approved for cutaneous T-cell lymphoma in the USA.⁶⁵ The biological response to HDACi includes the upregulation of p21 in a p53-independent fashion, which causes cell cycle arrest in the G₁ phase.⁶⁶ The HDACi butyrate and trichostatin A (TSA) have been shown to stabilize p21 mRNA, repress cyclins A and D, and activate p16 and p27, causing cell cycle arrest.^{67–69} Furthermore, certain HDACi increase the expression of proapoptotic genes, such as TRAIL, DR5, Bax, Apaf-1, Bmf, Bim and TP2 and/or downregulate antiapoptotic genes (such as BCL2, MCL1 and XIAP).^{67,70} Functionally, treatment with several HDACi also leads to prolonged IR-induced γ H2AX foci.^{71,72} This has been suggested to result, at least in part, owing to the impaired DDR signalling and repair following HDAC inhibition. Indeed, treatment with vorinostat [suberoyl + anilide + hydroxamic acid (SAHA)] together with IR leads to a reduction in levels of RAD50 following IR in melanoma cells or DNA-PK in prostate cells,⁷³ again suggesting compromised DNA repair. Down regulation of ATM gene expression and signalling has also been demonstrated in response to IR following HDAC inhibition.^{74,75}

As radiosensitizing agents, HDACi have shown promise *in vitro* in a variety of cell lines as well as in xenograft studies⁷⁶ (Table 3). Vorinostat has been demonstrated to enhance radiation-induced apoptosis at low micromolar concentrations in DU145 prostate cancer cells.⁷⁶ Vorinostat has also demonstrated effects as a radiosensitizer in melanoma, non-small-cell lung cancer, prostate, glioma, osteosarcoma, rhabdomyosarcoma and glioma cancers and cell lines.^{73,79–82,88} The HDAC inhibitor etinostat has shown radiosensitizing effects towards prostate cancer *in vitro*, and this effect was the greatest when the drug was present before and after radiation.⁸⁷ When erythroleukemic K562 cells were treated with valproic acid 24-h pre-radiation, the cells exhibited enhanced radiosensitivity, leading to increased radiation-induced cell death and apoptosis.⁸⁹ The HDACi valproic acid radiosensitizes colorectal cancer cell lines,⁷⁷ while TSA has been found to

Table 2. Reported radiosensitizing effects of selected DNA methyltransferase inhibitors

Inhibitor	Mechanism of radiosensitization	Cell lines tested	Reference
Decitabine	Prolonged γ H2AX expression	A549 and U373MG	Kim et al ⁵⁷
Azacitidine	Demethylation of p16 and MLH1 promoters observed in radiosensitive colorectal cell lines DNA damage response signalling and apoptosis induced in MM cells	HCT116, SW480, L174T, Co115 No radiosensitizing effect seen in A549 or U373MG MM.1S, MM.1R	Hofstetter et al, ⁵⁸ Kiziltepe et al ⁵⁹
Zebularine	Prolonged γ H2AX expression	A549 and U373MG	Kim et al ⁵⁷

MM, multiple myeloma.

Figure 2. The proposed mechanism of radiosensitization by DNA methyltransferase inhibitors (DNMTi). A schematic representation of the mechanism of action of DNMTi. The presence of a DNMTi following the generation of DNA damage by ionizing radiation (IR) results in hindered DNA repair and increased radiosensitivity.

radiosensitize cancers such as melanoma and squamous carcinoma.⁸⁵ The HDACi H6CAHA has demonstrated radiosensitizing effects against prostate cancer cell lines,⁷⁵ and the compound PC1-24781 radiosensitizes lung adenocarcinoma, large-cell lung

and colon cancer cell lines.⁸⁵ The HDACi Scriptaid (Karus Therapeutics, Pbingdon, UK) radiosensitizes squamous carcinoma cancer cell lines.⁸⁶

HISTONE METHYLTRANSFERASE INHIBITORS

The complex code of histone methylation, in addition to the role of these enzymes within the context of multiprotein complexes, makes these enzymes challenging to target therapeutically.²³ Despite current difficulties in targeting the HMTs, our knowledge of their function is rapidly increasing.^{47,90} As described, several histone methylation marks as well as some of the methyltransferases are emerging as facilitators of the DDR. Decreases in the levels of histone methylation on specific residues might therefore impair DDR signalling and lead to sensitization.⁴⁷ Since loss of ATM is well known to lead to exquisite radiosensitization, it is tempting to speculate that attenuating ATM signalling through targeting chromatin modifications essential for ATM activation may lead to similar effects, that is, increased radiosensitivity. Inhibition of H3K9me3, the chromatin mark associated with ATM activation, by loss of two important methyltransferases, Suv39h1 and Suv39h2, could therefore lead to radiosensitization. Chaetocin, a fungal metabolite that binds to the SAM binding site, is a currently available Suv39h1 and G9a inhibitor (Figure 3).⁹¹ G9a inhibitors such as BIX-01294, UNC0321, UNC0638 or UNC0646 are also available, but only limited pre-clinical testing has been carried out with these to date.^{23,92} As more and more specific inhibitors of the HMTs are developed in the future, it will be interesting to test them in combination with radiation.

Table 3. Reported radiosensitizing effects of selected histone deacetylase inhibitors

Inhibitor	Mechanism of radiosensitization	Cells lines tested	Reference
Valproic acid	Radiosensitization dependent on p53 (colorectal cell lines) Inhibition of DNA double-strand break repair and prolonged γ H2AX foci	LS174T, HCT116, K562, SF539 and U251	Chen et al, ⁷⁷ Camphausen et al ⁷⁸
Vorinostat	Appears to inhibit non-homologous end joining following radiation Increases γ H2AX foci formation and downregulates Ku70, Ku80, Ku86, DNA-PKcs, Rad51, EGFR, AKT in a variety of cell lines	A375, MeWo, A549, DU145, U373vIII, KHOS-24OS, SAOS2 (OS), A-204, RD (RMS), MDA-MB-231-BR, NB1691, SY5Y, Tet21 (MYCN overexpressed), Tet21 (MYCN repressed)	Munshi et al, ⁷³ Baschnagel et al, ⁷⁹ Karagiannis and El-Osta, ⁸⁰ Blattmann et al, ⁸¹ Mueller et al, ⁸² Karagiannis and El-Osta ⁸³
Trichostatin A (TSA)	Downregulates Ku700, Ku80 and DNA-PKcs Prolongs γ H2AX Enhances G ₂ /M cell cycle arrest and apoptosis (via mitochondrial pathway)	A549, H1650, A375, MeWo, SQ-20B, SCC-35	Munshi et al, ⁷² Zhang et al ⁸⁴
H6CAHA	Downregulates ATM, BRCA1 and BRCA2 Prolongs γ H2AX	PC3, DU145, LNCaP	Konsoula et al ⁷⁵
PCI-24781	Downregulates Rad51	HCT116, NCI-H460, A549	Adimoolam et al ⁸⁵
Scriptaid	Downregulates Ku80 (but not Ku70) Prolongs γ H2AX	SQ-20B	Kuribayashi et al ⁸⁶
Etinostat	Prolongs γ H2AX	DU145, U251	Camphausen et al ⁸⁷

Figure 3. The proposed mechanism of radiosensitization by the histone methyltransferase chaetocin. Regions of H3K9me3 are important for TIP60-dependent ATM acetylation, an event that facilitates ATM activation following DNA damage. Chaetocin inhibits H3K9me3 methyltransferases including Suv39h1 and may thereby reduce H3K9me3 levels, TIP60-dependent ATM acetylation and the subsequent ATM activation. Failure to activate ATM appropriately may lead to increased sensitivity to DNA damage induced by ionizing radiation. Me, methylation.

The mixed lineage leukaemia 1 (MLL) methyltransferase is associated with chromosomal translocations resulting in the expression of chimeric fusion proteins and deregulated recruitment of DOT1L. The DOT1L inhibitor, EPZ004777 (a SAM analogue) has been showed to be effective in cell lines and xenografts with MLL translocations.⁹³ Since DOT1L is known to be important for 53BP1 recruitment and would be predicted to impact NHEJ-mediated repair, the use of a DOT1L inhibitor could be envisioned to also be of therapeutic benefit in combination with radiation.²⁸

However, it is clear that more investigation is needed regarding the development of HMTi that is both highly selective and well tolerated before it will be possible to assess the full therapeutic potential of such treatments and their ability to act as radiosensitizers. Structural diversity of SAM binding sites amongst all methyltransferases suggests selectivity could be achieved.^{28,94,95}

HISTONE DEMETHYLASE INHIBITORS

A number of HDM inhibitors (HDMi) have been developed in recent years. The involvement of both LSD1 as well as some of the members of the JmjC family in DDR signalling suggests that targeting these may also impact the DDR and therefore, once again, could lead to radiosensitization if used in combination with IR. Currently available HDMi can be classified

according to their ability to inhibit the AOL domain-containing family or the JmjC demethylases. Current JmjC inhibitors compete with the cofactor (2-oxoglutarate) and bind iron in the active site.⁹⁵ Interestingly, HDACi have also displayed HDM inhibitory properties, and this overlap is thought to be owing to the shared enzymatic characteristics of co-ordinating metal ions. For example, SAHA has displayed moderate inhibition of JMJD2E but is much less effective as a JmjC inhibitor than as a HDACi.²⁰

CONCLUSION

As described above, a variety of chromatin remodellers and chromatin-remodelling processes have been shown to affect DNA damage sensing, signalling and repair. It is tempting to speculate that combining some of the inhibitors that have been developed to target these processes in combination with DNA damaging agents such as IR could be therapeutically beneficial. Indeed some of these compounds, such as HDACi, have already been shown to radiosensitize different tumour types at least in pre-clinical models. Before these approaches can be taken any further, the safety profiles of compounds will need to be investigated. For instance, the impaired DDR signalling and repair expected to result from the combination of some of these inhibitors with radiotherapy also raises the question of whether such combinations would also result in increased normal tissue toxicity. Nevertheless, it is important to bear in mind that in contrast to tumour cells, normal cells would not have compromised DNA repair pathways and may therefore be less affected by these combinations.^{96,97} Furthermore, differences in expression of chromatin remodelers between tumour and normal cells may also increase the tumour sensitivity towards therapeutic strategies targeting chromatin.^{67,68} Establishing a comprehensive safety profile will, however, be particularly important if inhibitors of chromatin modifiers that have also been implicated in tumour suppression are to be used in combination with radiotherapy (such as TIP60). The timing of administration of these inhibitors in relation to irradiation protocols as well as the choice of tumour type will also be critical in devising the most effective radiosensitizing strategies. It will be important to investigate whether the use of these compounds for short treatment courses, perhaps limited to just before or for the duration of radiation therapy, may alleviate some of the safety concerns. It is clear, however, that an area of novel and exciting possibilities is likely to emerge from the study of future therapeutic strategies involving the chromatin modulation together with radiotherapy.

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