Histone deacetylases support the accurate repair of broken forks

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ABSTRACT

We have recently uncovered that loss of the yeast histone deacetylases Rpd3 (Reduced Potassium Dependency 3) and Hda1 (Histone DeAcetylase 3) affects the cohesion between sister chromatids thus impairing repair of DNA damage at replication forks and enhancing genetic instability. Here we discuss the possible implications of our findings given that histone deacetylases are a promising chemotherapeutic target often used in combination with DNA damaging agents.

The basis for tumor origin and progression are genetic and epigenetic changes in the DNA. Histone acetylation is one of the best-studied epigenetic modifications and affects multiple cellular processes. Histone deacetylases (HDACs) remove acetyl groups from histones as well as from other non-histone targets. Importantly, HDAC levels appear altered in cancer cells, a number of HDAC inhibitors are used in clinical trials and some of them are already approved by the Food and Drug Administration and used as anticancer agents [1]. In particular, preclinical and clinical trials have nurtured high expectations in the use of HDAC inhibitors as chemosensitizers that increase the efficiency of other chemotherapeutic compounds or radiotherapy. Understanding the effects of HDAC loss is therefore crucial in order to fully evaluate the possible consequences of therapies using HDAC inhibitors, particularly under conditions of DNA damage and in tumoral cells. In a study performed in the yeast *S. cerevisiae*, we have recently shown that the loss of different subunits form the HDAC complexes Rpd3L (Reduced Potassium Dependency 3, Large) and Hda1 (Histone DeAcetylase 3), which catalytic subunits are Rpd3 and Hda1, related to human class I and IIB HDACs respectively, causes a strong defect in the repair of broken forks leading to genetic instability [2] (Figure 1).

Replication forks can collapse into double-strand breaks (DSBs) as a consequence of encountering different sources of obstacles, particularly in the absence of an optimal DNA Damage Response (DDR), which is frequently deficient in cancer cells and consequently considered an anticancer barrier. Replication forks will frequently collapse after encountering nicks in the DNA leading to DSBs. These are estimated to happen at the highest frequency among all DNA lesions reaching up to 55000 per cell per day [3]. Two main pathways, non-homologous end joining and homologous recombination, can repair DSBs. However, broken replication forks are preferentially repaired by homologous recombination in a reaction that uses the intact sister chromatid as a template (Sister Chromatid Recombination, SCR). Using a powerful-engineered system in which nicks are induced by the action of the yeast endonuclease HO into an incomplete target sequence placed in a reporter gene, we have shown that mutants of the yeast Rpd3 and Hda1 HDACs strongly impair SCR [2]. Furthermore, we have improved the system by substituting the endonuclease HO by a modified version of the flipase so that nicks can be specifically targeted to a flipase recognition site located on either DNA strand. This has enabled us to see that the kinetics of repair of broken forks with the sister chromatid is similar regardless of whether the nick was induced in the leading or lagging strand.

Interestingly, HDACs affect SCR because they are required for the proper loading of cohesins [2]. Cohesin is a conserved multi-subunit protein complex that hold the sister chromatids together thus enabling proper SCR, as shown in yeast and human cells [4,5]. Cohesion is established during replication and lasts until the onset of mitosis to enable the separation of the sister chromatids, but additional cohesins have been shown to load upon DNA damage independently of replication [6]. So far, the role of cohesins in

promoting SCR was attributed to this damage-induced cohesion. However, Rpd3 and Hda1 loss affect the general loading of cohesins regardless of the induction of DNA damage, supporting that general cohesion, and not only damage-induced cohesion, is crucial for efficient repair by SCR [2]. The cohesion dynamic cycle itself is regulated by acetylation of the SMC3 (Structural maintenance of chromosomes protein 3) cohesin component, which is deacetylated by the human HDAC8 class I HDAC, related to yeast Rpd3. However, a deletion mutant in the equivalent yeast HDAC, Hos1 (Hda One Similar 1), which performs this function instead of Rpd3, entails no defect in SCR [2]. This argues that the role of Rpd3 and Hda1 in cohesion is actually executed by the deacetylation of histones and not by the deacetylation of cohesins themselves. The specific histone residues that need to be deacetylated and the exact mechanism by which acetylation affects cohesion remains to be elucidated.

Strikingly, the loss of Rpd3 and Hda1 enhances the probabilities of recombinational repair with ectopic templates rather than the intact sister chromatid and the defect in SCR can ultimate lead to other genomic rearrangements such as chromosome loss or gross chromosomal rearrangements [2]. Still, histone deacetylation can also have negative effects at the fork since it has been reported that Rpd3 is responsible for the DNA damage sensitivity of checkpoint mutants [7]. Although the effect of HDAC loss in SCR in human cells still needs to be properly addressed, so far HDAC inhibitors have been shown to impair timely DNA damage repair, particularly in cancer cells [8]. Hence, HDAC inhibition in human cells would be also expected to enhance genetic instability. Moreover, genetic instability would arise in the absence of HDAC inhibitors due to multiple mechanisms, since it has been shown that they also enhance the formation of harmful DNA-RNA hybrids that block replication fork progression [9]. The increased genetic instability might justify not only the enhanced toxicity but also some of the side effects of HDAC inhibitors in combined chemotherapy, particularly when applied in combination with DNA damaging agents. Indeed, artificially diminishing the expression of the *RAD21* gene (RADiation sensitive 21), which encodes for one of the cohesin components, was reported to enhance the cytotoxicity of the DNA damaging agents etoposide and bleomycin in cancer cells [10]. A similar effect would be expected by HDAC inhibitors that affect SCR through cohesion impairment. Moreover, it is worthy of note that the genetic instability caused by HDAC inhibition might also contribute to the development of new tumoral processes or chemoresistance by promoting the acquisition of extra mutations. In summary, our recent results [2] demonstrate that HDAC levels are crucial for genome integrity by ensuring the proper repair of DSBs at replication forks, which might occasionally occur in all cells and are likely more frequent in cancer cells.

KEYWORDS: histone deacetylases; histone deacetylase inhibitors; double-strand break repair; cohesion; sister-chromatid recombination

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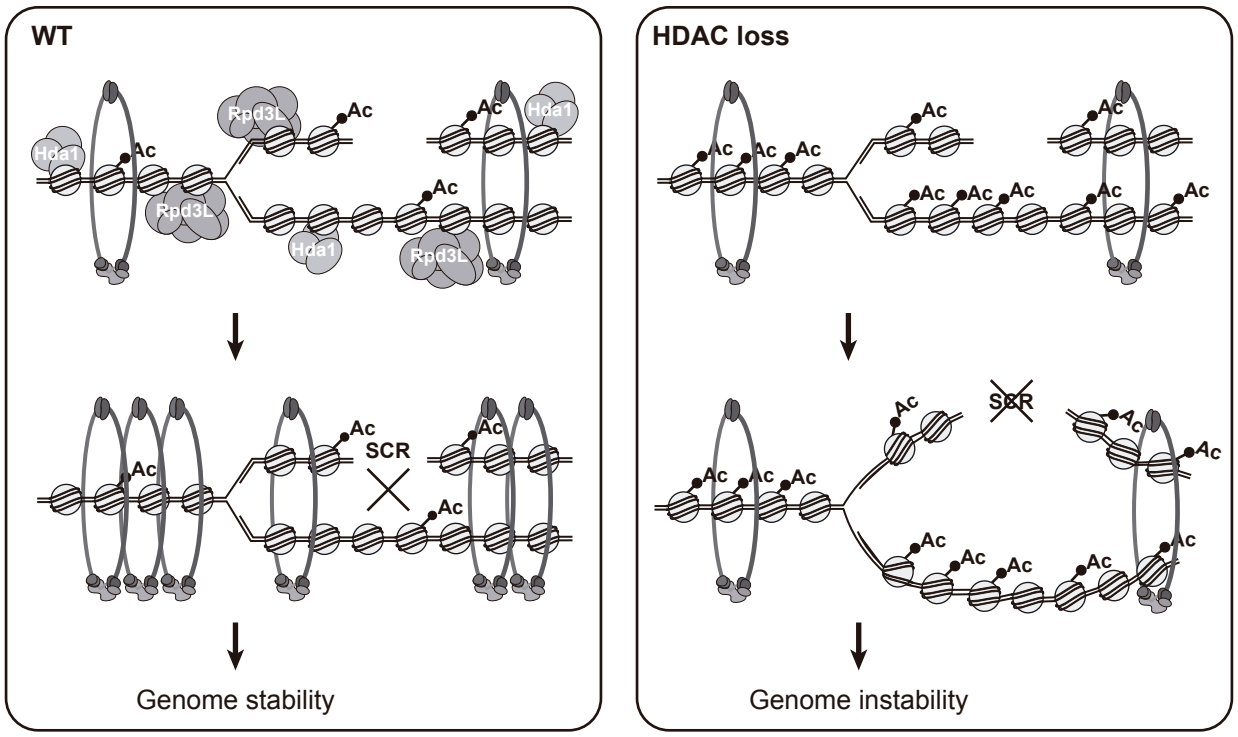
REFERENCES

1. Suraweera, A., O'Byrne, K.J. & Richard, D.J. Combination Therapy With Histone Deacetylase Inhibitors (HDACi) for the Treatment of Cancer: Achieving the Full Therapeutic Potential of HDACi. Front Oncol 2018;8:92.
2. Ortega, P., Gomez-Gonzalez, B. & Aguilera, A. Rpd3L and Hda1 histone deacetylases facilitate repair of broken forks by promoting sister chromatid cohesion. Nat Commun 2019;10:5178.
3. Lindahl, T. & Barnes, D.E. Repair of endogenous DNA damage. Cold Spring Harb Symp Quant Biol 2000;65:127-133.
4. Cortes-Ledesma, F. & Aguilera, A. Double-strand breaks arising by replication through a nick are repaired by cohesin-dependent sister-chromatid exchange. EMBO Rep 2006;7:919-926.
5. Potts, P.R., Porteus, M.H. & Yu, H. Human SMC5/6 complex promotes sister chromatid homologous recombination by recruiting the SMC1/3 cohesin complex to double-strand breaks. EMBO J 2006;25:3377-3388.
6. Sjogren, C. & Strom, L. S-phase and DNA damage activated establishment of sister chromatid cohesion--importance for DNA repair. Exp Cell Res 2010;316:1445-1453.
7. Gomez-Gonzalez, B., Patel, H., Early, A. & Diffley, J.F.X. Rpd3L Contributes to the DNA Damage Sensitivity of Saccharomyces cerevisiae Checkpoint Mutants. Genetics 2019;211:503-513.
8. Lee, J.H., Choy, M.L., Ngo, L., Foster, S.S. & Marks, P.A. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. Proc Natl Acad Sci USA 2010;107:14639-14644.
9. Salas-Armenteros I, Pérez-Calero C, Bayona-Feliu A, Tumini E, Luna R, Aguilera A. Human THO-Sin3A interaction reveals new mechanisms to prevent R-loops that cause genome instability. EMBO J. 2017;36:3532-3547.
10. Atienza, J.M., Roth, R.B., Rosette, C., Smyllie K.J., Kammerer, S., Rehbock, J., Ekblom, J., Denissenko, M. F. Suppression of RAD21 gene expression decreases cell growth and enhances cytotoxicity of etoposide and bleomycin in human breast cancer cells. Mol Cancer Ther 2005;4:361-368.

FIGURE LEGEND

Figure 1. Histone deacetylases facilitate the accurate repair of broken forks by promoting sister chromatid cohesion. In wild-type cells (WT), the removal of acetyl groups (Ac) from histones by the histone deacetylases (HDACs) complexes Rpd3L (Reduced Potassium Dependency 3, Large) and Hda1 (Histone DeAcetylase 3) promotes the cohesion that entraps the two sister chromatids to the levels required for efficient repair of replication-born double strand breaks with the sister chromatid (Sister-Chromatid Recombination, SCR). In the absence of any of these HDACs, inefficient cohesin loading causes an impairment of SCR and increased levels of genome instability (*modified from* ref. 2).

Figure 1


WT
Hda1
Ac
Rpd3L
Ac
Ac
Hda1
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Rpd3L
Hda1
Rpd3L
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SCR
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HDAC loss
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