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0–24 h, as we have previously shown that this TSA concentration sensitizes HCT116 cells to ionizing radiation[3]. We observed rapid induction of H3 and H4 hyperacetylation in all the cell lines, irrespective of HDAC2 status. These responses were transient, and baseline histone acetylation status was restored after 18–24 h of TSA incubation (**Fig. 1b** and **Supplementary Fig. 2** online). Further, we observed almost identical time-dependent hyperacetylation of H3 and H4 in RKO-ES cells incubated with 250 nM TSA (**Supplementary Fig. 3** online). Finally, radiocytotoxicity was amplified by TSA in both RKO-ES and RKO-ATCC cell lines (**Fig. 1c**), essentially as we previously demonstrated in the HCT116 cell line[3].

Currently, a dozen HDAC inhibitors are under investigation in clinical trials[5,6], and predictive molecular markers reflecting therapeutic effect are warranted. As suggested by Ropero *et al.*[4], HDAC2 deficiency might impair the therapeutic response to HDAC inhibitors. However, their contention that HDAC2 deficiency confers resistance to TSA-induced histone acetylation could not be confirmed by our results, as transient hyperacetylation of H3 and H4 was detected in the HDAC2-defective cell lines upon TSA treatment. We believe that hyperacetylation of H3 and H4, at least in the examined model systems, is not solely dependent on functional HDAC2. Furthermore, in a model system of radiosensitization regulated by HDAC inhibition, TSA was equally effective in RKO cells regardless of whether they were deficient or proficient in HDAC2, suggesting that loss of HDAC2 function may not alter tumor response to HDAC inhibitors in radiotherapy. Consequently, tumor HDAC2 status should not need to be taken into account when investigating HDAC inhibitors as radiosensitizers.

***Anne Hansen Ree*[1,2], *Sigurd Folkvord*[1,3] & *Kjersti Flatmark*[1,3,4]**

[1]Department of Tumor Biology, Norwegian Radium Hospital, Rikshospitalet University Hospital, 0310 Oslo, Norway. [2]Faculty Division Akershus University Hospital, and [3]Faculty Division The Norwegian Radium Hospital, Faculty of Medicine, University of Oslo, 0318 Oslo, Norway. [4]Department of Surgical Oncology, Norwegian Radium Hospital, Rikshospitalet University Hospital, 0310 Oslo, Norway. Correspondence should be addressed to A.H.R. (a.h.ree@medisin.uio.no).

Note: Supplementary information is available on the Nature Genetics website.

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1. Cerna, D. *et al. Curr. Top. Dev. Biol.* **73,** 173–203 (2006).
2. Nome, R.V. *et al. Mol. Cancer Ther.* **4,** 1231–1238 (2005).
3. Flatmark, K. *et al. Radiat. Oncol.* **1,** 25 (2006).
4. Ropero, S. *et al. Nat. Genet.* **38,** 566–569 (2006).
5. Kelly, W.K. & Marks, P.A. *Nat. Clin. Pract. Oncol.* **2,** 150–157 (2005).
6. Bolden, J.E. *et al. Nat. Rev. Drug Discov.* **5,** 769–784 (2006).

Ropero and Esteller reply: We are delighted to learn that Ree *et al.* have confirmed our results showing the presence of inactivating mutations in *HDAC2* that lead to loss of HDAC2 protein in Co115 colorectal cancer cells[1]. We are also glad that these same authors have validated our finding of the presence of a truncating mutation in *HDAC2* that results in loss of HDAC2 protein in the original RKO colorectal cancer cells used in our study[1]. The only minor difference we can see between our study and that of Ree *et al.* is that, using another batch of RKO cells, they observed only a reduction of HDAC2 protein, whereas we observed minimal expression. The minimal expression of the HDAC2 protein in RKO mutant cells has also been confirmed by others (J.G. Herman, Johns Hopkins Medical Institutions, personal communication). We analyzed 700 SNPs and found complete identity between the original RKO cells and a recent batch obtained from the American Type Culture Collection (data not shown). Ree *et al.* did not provide their cells for further tests.

Another interesting issue is the response to hydroxamic HDAC inhibitors, such as trichostatin A (TSA), according to the HDAC2 mutational status. We observed a reduced biochemical and cellular response to these drugs in cells that harbor the mutation in *HDAC2* (ref. 1). A similar resistance to HDAC inhibitor–induced apoptosis has been found in another set of independent RKO HDAC2 mutant cells (J.G. Herman, personal communication). Ree *et al.* try to suggest that histone acetylation upon TSA treatment might be independent of HDAC2 mutational status. The problem is that the histone acetylation profiles induced by TSA administration provided by Ree *et al.* contradict the previously published data from these same authors based on the same colorectal cancer cell lines, drug and conditions[2]. This inconsistency precludes drawing any further valid conclusion from their data.

Overall, the body of data from these groups and others[3] and our recent finding that HDAC2 impairment leads to aberrant gene expression[4] support the presence of *HDAC2*-inactivating mutations in a subset of unstable microsatellite human tumors that renders these cells more resistant to the usual antiproliferative and proapoptotic effects of hydroxamic-based histone deacetylase inhibitors.

Santiago Ropero & Manel Esteller

Cancer Epigenetics Group, Spanish National Cancer Research Centre, 28029 Madrid, Spain. Correspondence should be addressed to M.E. (mesteller@cnio.es).

1. Ropero, S. *et al. Nat. Genet.* **38,** 566-569 (2006).
2. Flatmark, K. *et al. Radiat. Oncol.* **1**, 25 (2006).
3. Ozdağ, H. *et al. BMC Genomics* **7**, 90 (2006).
4. Ropero, S. *et al. Oncogene* advance online publication, doi:10.1038/onc.2008.31 (11 February 2008).