

Unraveling a Connection between DNA Demethylation Repair and Cancer

Manel Camps¹ and Brandt F. Eichman^{2,*}

¹Department of Microbiology and Environmental Toxicology, University of California Santa Cruz, 1156 High Street, Santa Cruz, CA 95060, USA

²Department of Biological Sciences, Vanderbilt University, 465 21st Avenue South, Nashville, TN 37232, USA

*Correspondence: brandt.eichman@vanderbilt.edu

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In this issue of *Molecular Cell*, Dango and Mosammaparast discover that the human oxidative demethylase ALKBH3 functions in complex with a DNA helicase to eliminate N3-methylcytosine lesions from ssDNA and that specific cancer cell lines are dependent on this activity for proliferation (Dango et al., 2011).

The endogenous methyl donor, S-adenosylmethionine, and exogenous alkylating agents react nonenzymatically with DNA nucleobases to generate toxic and mutagenic alkyl adducts, which lead to heritable diseases and genome instability. Particularly deleterious are modifications to Watson-Crick base-pairing positions, since they can interfere with replication, transcription, and translation. The most common of these lesions are N1-methyladenine (1mA) and N3-methylcytosine (3mC), which in humans are repaired mainly by two demethylases: ALKBH2 and ALKBH3 (Sedgwick et al., 2007). The mechanisms by which these enzymes integrate into cellular DNA transactions to maintain integrity of the genome are largely unknown. In this issue, Dango and Mosammaparast discover that repair of 3mC in vivo depends on a collaboration between ALKBH3 and a DNA helicase and establish an important link between overexpression of ALKBH3 and cancer (Dango et al., 2011).

A decade ago, *E. coli* AlkB was found to repair 1mA and 3mC by oxidative demethylation, an error-free, single-step enzymatic reaction that removes the methyl group to restore the original base (Figure 1A) (Falnes et al., 2002; Trewick et al., 2002). AlkB homologs (ALKBH) were subsequently found in multicellular organisms, underscoring the fundamental importance of this repair mechanism for genomic integrity (Sedgwick et al., 2007). Humans contain 9 ALKBH genes, three of which—ALKBH1, ALKBH2, and ALKBH3—are known to perform in vitro DNA demethylation analogous to AlkB. Whereas ALKBH2 is specific for dsDNA,

ALKBH3 has a preference for single-strand templates and is the only oxidative demethylase known to repair both DNA and RNA substrates with similar efficiency in vitro (Sedgwick et al., 2007). Importantly, alterations in expression of ALKBH2, 3 and 8 have been described in diverse tumors, and ALKBH2 and ALKBH3 expression has been linked to positive effects on proliferation of tumor-derived cell lines, providing indirect evidence for a possible role of these genes in DNA homeostasis (Gao et al., 2011; Tasaki et al., 2011). In mice, however, ALKBH3 appeared to be dispensable for 1mA and 3mC repair, questioning the role of this enzyme in genome maintenance (Ringvoll et al., 2006).

In an effort to address the relevance of ALKBH3 in mammalian cells, Dango, Mosammaparast, and colleagues make the significant discovery that ALKBH3 physically associates with a 3'-5' DNA helicase known as the activating signal cointegrator complex subunit 3 (ASCC3), and show that ASCC3's DNA unwinding activity provides the ssDNA substrate for ALKBH3 (Figure 1B) (Dango et al., 2011). Thus, coupling ALKBH3 repair and ASCC3 DNA unwinding activities enables ALKBH3 to process 3mC within double-stranded regions of DNA. The ALKBH3-ASCC3 complex, like those of other DNA-repair enzymes, may also serve to recruit additional repair factors to sites of DNA damage or to establish crosstalk between multiple pathways involved in genome maintenance. In fact, ALKBH2 was previously shown to associate with PCNA (Gilljam et al., 2009), suggesting that partnership with proteins involved in

DNA transactions may be a general feature of the ALKBH family.

Interestingly, the ASCC complex was previously characterized as a transcription coactivator of nuclear receptors, raising the possibility that ALKBH3 demethylation may be coupled to transcription and/or chromatin remodeling. Indeed, the superfamily 2 (SF2)-type helicases to which ASCC3 belongs are typically involved in chromatin remodeling through their ATP-dependent DNA translocase activities. It is interesting to note that *E. coli* AlkB has been postulated to play a role in transcription-coupled DNA repair (TCR), which is initiated by the multifunctional SF2 helicase, Mfd (Wrzesiński et al., 2010). It is tantalizing to speculate that AlkB also functions in conjunction with a helicase given its substrate preference for ssDNA. Furthermore, while the ALKBH3-ASCC3 interaction nicely explains the preference of ALKBH3 for ssDNA, the rationale for its activity toward RNA substrates is not as clear given the generally high turnover rate of RNA. A link to transcription may serve to explain this dilemma.

In the current work, knockdown of either ALKBH3 or ASCC3 in specific tumor cell lines led to an increase in 3mC adducts and to the formation of γ H2AX and 53BP1 foci, consistent with activation of the DNA-damage response. Most significantly, loss of ALKBH3 and ASCC3 significantly reduced cell proliferation in these cell lines (Dango et al., 2011). This observation suggests that ALKBH3 acts as a “gatekeeper” to prevent 3mC accumulation in tumors and raises the exciting possibility that inhibition of ALKBH3,

either alone or in combination with DNA-damaging agents, may be of therapeutic value for cancer treatment. In contrast, loss of ALKBH2 in gastric tumors was reported by another study to result in increased proliferation (Gao et al., 2011), suggesting an additional role of ALKBH2 as a replication checkpoint, possibly through its interaction with PCNA.

The present work proves that generation of 3mC from endogenous sources can limit cell proliferation, in agreement with the observation that 3mC is generally the preferred substrate in prokaryotic AlkB genes (van den Born et al., 2009). Strong alkylating agents currently in use in chemotherapy preferentially target purine N3 and N7 positions in dsDNA, whereas the base-pairing positions in dsDNA are shielded from alkylation (Sedgwick et al., 2007). New methylating agents favoring the generation of 3mC in ssDNA could be developed to exploit the interplay between ALKBH3 and 3mC levels described here. The ALKBH3 dependence on cell proliferation is restricted to a small subset of tumors that show ALKBH3 and ASCC3 overexpression, and thus new therapeutic approaches based on 3mC cytotoxicity will likely need to be customized to individual tumors. Tumor profiling may be facilitated by the availability of 3mC-specific antibodies and expression markers described in this work. It will be of interest to uncover the mechanisms responsible

for the tumor-type specificity of ALKBH3 dependence and for the associated ALKBH3 and ASCC3 upregulation, as these represent potential targets for therapeutic intervention and tumor stratification.

In summary, this article greatly advances our understanding of the role of ALKBH enzymes by furnishing a mechanistic understanding of ALKBH3 function *in vivo*, in the context of both genome

maintenance and transformation. This work also has important translational implications, identifying new, potential therapeutic approaches for the treatment of cancer and providing tools to stratify tumors according to 3mC repair status.

REFERENCES

- Dango, S., Mosammaparast, N., Sowa, M.E., Xiong, L.-J., Wu, F., Park, K., Rubin, M., Gygi, S., Harper, J.W., and Shi, Y. (2011). *Mol. Cell* **44**, this issue, 373–384.
- Falnes, P.O., Johansen, R.F., and Seeberg, E. (2002). *Nature* **419**, 178–182.
- Gao, W., Li, L., Xu, P., Fang, J., Xiao, S., and Chen, S. (2011). *J. Gastroenterol. Hepatol.* **26**, 577–584.
- Gilljam, K.M., Feyzi, E., Aas, P.A., Sousa, M.M., Müller, R., Vågbo, C.B., Catterall, T.C., Liabakk, N.B., Slupphaug, G., Drablos, F., et al. (2009). *J. Cell Biol.* **186**, 645–654.
- Ringvoll, J., Nordstrand, L.M., Vågbo, C.B., Talstad, V., Reite, K., Aas, P.A., Lauritzen, K.H., Liabakk, N.B., Bjørk, A., Doughty, R.W., et al. (2006). *EMBO J.* **25**, 2189–2198.
- Sedgwick, B., Bates, P.A., Paik, J., Jacobs, S.C., and Lindahl, T. (2007). *DNA Repair (Amst.)* **6**, 429–442.
- Tasaki, M., Shimada, K., Kimura, H., Tsujikawa, K., and Konishi, N. (2011). *Br. J. Cancer* **104**, 700–706.
- Trewick, S.C., Henshaw, T.F., Hausinger, R.P., Lindahl, T., and Sedgwick, B. (2002). *Nature* **419**, 174–178.
- van den Born, E., Bekkelund, A., Moen, M.N., Omelchenko, M.V., Klungland, A., and Falnes, P.O. (2009). *Nucleic Acids Res.* **37**, 7124–7136.
- Wrzesiński, M., Nieminuszczy, J., Sikora, A., Mielecki, D., Chojnacka, A., Kozłowski, M., Krwawicz, J., and Grzesiuk, E. (2010). *Mutat. Res.* **688**, 19–27.

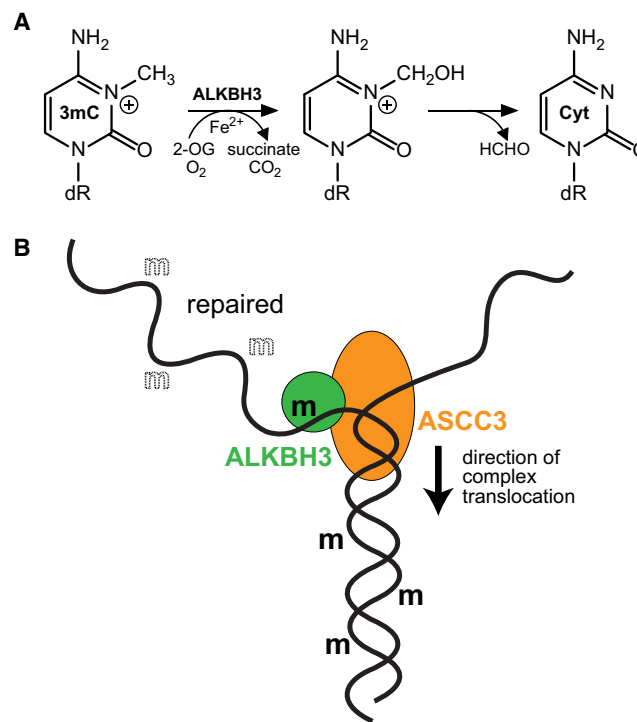


Figure 1. Repair of N3-Methylcytosine (3mC) by Human ALKBH3

(A) ALKBH3 catalyzes the oxidative demethylation of 3mC. In an Fe(II)-dependent reaction, the N3-methyl group is hydroxylated by molecular oxygen to yield an N3-hydroxymethyl intermediate, from which formaldehyde is spontaneously released to produce cytosine (Cyt) base. A 2-oxoglutarate (2-OG) cosubstrate is converted to succinate and carbon dioxide in the process. (B) ALKBH3 repairs 3mC lesions (bold **m**) from ssDNA by forming a complex with the ASCC3 helicase. 3mC is frequently found in dsDNA, which is unwound by ASCC3 to provide ALKBH3 with its preferred ssDNA substrate. In the figure, a clear *m* denotes a 3mC lesion that has been demethylated to cytosine.