form structures ranging from monoliths to meshes to open vessels (*4*). Multiple glass-forming compositions can be mixed inline to create spatial composition variation, such as gradient refractive index glass lenses (*5*). Stereolithography printing—a layer-by-layer additive manufacturing route by which photocurable nanocomposite liquid resins are selectively patterned by exposure to light—can produce transparent glass components that have complex and hierarchical structures with the potential for fine-scale individual features (*6*–*9*).

Most recently, Mader *et al.* have demonstrated the use of pelletized glass-forming composites that are compatible with conventional, low-temperature injection molding—a process used in the high-volume manufacture of polymeric components. Offering an important advance toward sustainable manufacturing, much of the debinding occurs in water rather than at increased temperatures, and the polymers used in the composite can be reclaimed and reused. Fused silica glass items, including tubes, beakers, and microlens arrays, were produced with this composite and existing injection molding equipment at speeds of up to 5 s per piece, without any postprocessing after sintering.

These approaches could make glass available in new formats as well as more cost-competitive with plastic for applications requiring thermal stability, environmental resistance, or improved light transmission. Distributed manufacturing may become a more viable option because processing is done at low temperatures with less-specialized equipment. Additive manufacturing allows changes in component shape through programming rather than retooling for small-volume manufacture and rapid prototyping of glass components. Immediate applications in high-volume optics, lighting, and packaging will drive continued development in low-temperature processing. The combination of methods that enable tunable glass composition and hierarchical microstructures could find use in microfluidics and catalysis. ■


### REFERENCES AND NOTES

1. M. Mader *et al.*, *Science* **372**, 182 (2021).
2. L. L. Hench, J. K. West, *Chem. Rev.* **90**, 33 (1990).
3. F. Kotz *et al.*, *Adv. Mater.* **30**, 1707100 (2018).
4. D. T. Nguyen *et al.*, *Adv. Mater.* **29**, 1701181 (2017).
5. R. Dylla-Spears *et al.*, *Sci. Adv.* **6**, eabc7429 (2020).
6. F. Kotz *et al.*, *Nature* **544**, 337 (2017).
7. I. Cooperstein, E. Shukrun, O. Press, A. Kamyshny, S. Magdassi, *ACS Appl. Mater. Interfaces* **10**, 18879 (2018).
8. R. Arita *et al.*, *Commun. Mater.* **1**, 30 (2020).
9. D. G. Moore, L. Barbera, K. Masania, A. R. Studart, *Nat. Mater.* **19**, 212 (2020).



### ACKNOWLEDGMENTS

This work was performed under the auspices of the US Department of Energy by Lawrence Livermore National Laboratory under contract no. DE-AC52-07NA27344.

10.1126/science.abg7657


CANCER

# Epigenetic nucleotides enhance therapy

## Targeting sanitation of epigenetic nucleotides synergizes with cancer treatment

*By* **Skirmantas Kriaucionis**

Targeted cancer therapy often relies on inhibiting beneficial adaptations of tumor cells. For example, inhibiting complementary or compensatory mechanisms can push cells over the edge of survival. An example of this kind of intervention, which is used in the clinic, is inhibition of poly(adenosine diphosphate–ribose) polymerase (PARP) for treatment of tumors with compromised DNA repair by the homologous recombination pathway (mutated *BRCA1* or *BRCA2* genes). Despite overall beneficial response to treatment with PARP inhibitors (PARPi), resistance is still a formidable problem. Although the search for PARPi sensitizers has been extensively explored, new combinations to improve responses are needed. On page 156 of this issue, Fugger *et al.* (*1*) found that interference with nucleotide metabolism potentiates the efficacy of PARPi in homologous recombination–compromised cancer cells. This discovery opens several promising avenues to enhance PARPi efficacy and could even hold promise for overcoming acquired resistance.

Although the lifetime risk of developing breast cancer in women is 13%, carrying a germline mutation in *BRCA1* or *BRCA2* genes increases this risk to ~70% (*2*). Moreover, mutations in these genes have been linked to increased risk of ovarian cancer and, more recently, prostate, colorectal, stomach, pancreatic, and other cancers. The primary function of the BRCA proteins is the repair of double-stranded DNA breaks by high-fidelity mechanisms using homologous recombination. Defects in homologous recombination lead to genomic instability and gross chromosomal rearrangements, which are typically observed in tumors and cell lines with compromised *BRCA1* or *BRCA2*. The identification of PARP involvement in DNA repair and development of PARPi, which both inhibit and trap PARP on DNA, led to observations that *BRCA1*- and *BRCA2*-deficient cells are hypersensitive to PARPi (*3*, *4*). Several PARPi were approved for treatment of ovarian and breast cancer, achieving a milestone especially in the treatment of ovarian cancer.

Fugger *et al.* undertook a genome-wide loss-of-function screen in haploid human leukemia cells to identify genes that cause resistance or increased sensitivity to the PARPi olaparib. Two genes involved in nucleotide metabolism, 2′-deoxynucleoside 5-monophosphate N-glycosidase (*DNPH1*) and inosine triphosphatase (*ITPA*), enhanced olaparib cytotoxicity. DNPH1 cleaves glycosidic bonds in deoxynucleotide monophosphates (MPs), releasing sugar monophosphate and nucleobase (*5*), whereas ITPA hydrolyzes inosine triphosphate (ITP), resulting in inosine MP (*6*). The substrates of DNPH1 were thought to be canonical nucleotide MPs. Examination of DNA composition in *DNPH1*-deficient cells identified accumulation of 5-hydroxymethyl-uracil base (hmU) in the DNA, suggesting that the free nucleotide 5-hydroxymethyl-deoxyuridine MP (hmdUMP) could be the best substrate of DNPH1. Fugger *et al.* also noticed that exposure to the nucleotide 5-hydroxymethyl-deoxycytidine (hmdC) has a similar synergy with PARPi. In this case, enzymatic deamination converts hmdCMP into hmdUMP in the nucleotide pool, which in turn potentiates activity of PARPi (see the figure). Previous work demonstrated that hmdC is not efficiently phosphorylated but is instead deaminated, ending up in DNA as hmdU (*7*).

Fugger *et al.* propose that the source of hmdU in nucleotide pools could be the epigenetic DNA base hmC. hmC is a product of ten-eleven translocation (TET) enzymes, which are involved in the oxidation of 5-methylcytosine at CpG sites in the DNA (*8*). hmC is not actively removed from DNA; however, it can be further oxidized by TET enzymes, resulting in engagement of base excision DNA repair pathway mediated demethylation (*9*). This could release hmdCMPs into the nucleotide pool. Alternatively, off-target activity of TET enzymes on thymine bases was reported to generate hmU in the DNA directly (*10*). Repair of hmU DNA base could also contribute to hmdUMP in the nucleotide pool.

How does accumulation of hmU in the DNA cause lethality in combination with PARPi? Fugger *et al.* show that uracil DNA


Ludwig Institute for Cancer Research, University of Oxford, Oxford, UK. E-mail: skirmantas.kriaucionis@ludwig.ox.ac.uk

glycosylase 1 (SMUG1), which removes hmU from DNA, generates abasic sites, which cause replication fork collapse that induce double-stranded DNA breaks and thereby provide additional sites for PARP trapping and enhance efficacy of PARPi. Other mechanisms that generate abasic sites should also synergize with PARPi. Indeed, it is likely that inhibiting other noncanonical nucleotide hydrolases, such as MutT homolog 1 (MTH1) and MTH2, might synergize with PARPi.

The discovery of hmdUMP as the preferred substrate for DNPH1 assigns a previously unknown nucleotide "sanitizer" role to this enzyme. Nucleotide "sanitation" involves the removal of potentially damaging, noncanonical nucleotide triphosphate variants from the nucleotide pool. In contrast to DNA, the nucleotide pool has higher susceptibility to damage because of more accessible reactive groups. Moreover, there is a flux of damaged bases and nucleosides from DNA repair and the extracellular space. To limit the availability of noncanonical nucleotides to DNA polymerases, cells have a broad group of enzymes consisting of Nudix hydrolases (with 24 characterized members), deoxyuridine triphosphatase (dUTPase), inosine triphosphatases (ITPases), and SAM domain and HD domain–containing protein 1 (SAMHD1) (*11*).

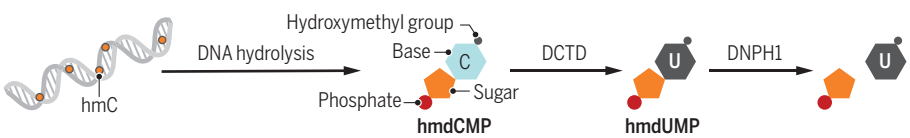
Future translational work should focus on developing DNPH1 inhibitors into drugs. It will also be interesting to explore inhibition of ITPA as a strategy to potentiate PARPi. Deletion of ITPA produced a higher increase of inosine in the genome than the amount of hmU accumulation observed after ablation of DNPH1, suggesting that ITPA inhibition might be even more effective in synergizing with PARPi. It will also be essential to use in vivo tumor models because maintaining similar efficacy while transitioning from cell lines to actual tumors is often complicated. Elucidation of the impact of DNPH1 or ITPA inhibition in normal cells will help to determine whether off-target effects are likely.

It will be important to know whether tumors have higher pools of hmdUMP or dITP, which could make them more susceptible to treatment with appropriate inhibitors. Multiple reports documented depleted hmC in DNA of cancer cells when compared with normal tissue. However, the interpretation of this observation in inferring hmdU abundance in the nucleotide pool is not straightforward because it may reflect either lower production of hmC by TET enzymes or enhanced removal of this base from DNA. ■

## Exploiting nucleotide sanitation

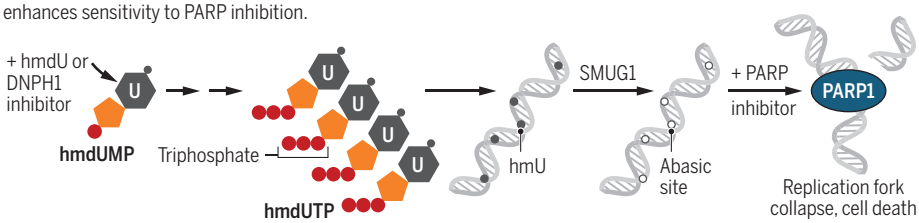
**Removal of hmdUMP from the nucleotide pool**

5-hydroxymethyl-cytosine (hmC) in DNA is released as the free nucleotide 5-hydroxymethyl-deoxycytidine monophosphate (hmdCMP). This is deaminated by deoxycytidylate deaminase (DCTD) to 5-hydroxymethyl deoxyuridine monophosphate (hmdUMP) and cleaved by 2′-deoxynucleoside 5-phosphate N-hydrolase 1 (DNPH1), resulting in its removal from the nucleotide pool.




**Opportunity for the therapy**

Administration of hmdU nucleoside and/or DNPH1 inhibitor leads to accumulation of hmdUMP in nucleotide pools, where it can be incorporated into DNA. This leads to 5-hydroxymethyluracil (hmU) in DNA and repair by uracil DNA glycosylase 1 (SMUG1), which generates abasic sites that can cause replication fork collapse and double-stranded DNA breaks that require poly(adenosine diphosphate–ribose) polymerase (PARP) for repair. Inhibition of DNPH1 thereby enhances sensitivity to PARP inhibition.





### REFERENCES AND NOTES

1. K. Fugger *et al.*, *Science* **372**, 156 (2021).
2. K. B. Kuchenbaecker *et al.*, *JAMA* **317**, 2402 (2017).
3. H. Farmer *et al.*, *Nature* **434**, 917 (2005).
4. H. E. Bryant *et al.*, *Nature* **434**, 913 (2005).
5. Y. K. Ghiorghi *et al.*, *J. Biol. Chem.* **282**, 8150 (2007).
6. S. Lin *et al.*, *J. Biol. Chem.* **276**, 18695 (2001).
7. M. Zauri *et al.*, *Nature* **524**, 114 (2015).
8. M. Tahiliani *et al.*, *Science* **324**, 930 (2009).
9. T. Pfaffeneder *et al.*, *Nat. Chem. Biol.* **10**, 574 (2014).
10. Y.-F. He *et al.*, *Science* **333**, 1303 (2011).
11. S. G. Rudd *et al.*, *DNA Repair* (Amst.) **44**, 193 (2016).



### ACKNOWLEDGMENTS

S.K. is supported by the Ludwig Institute for Cancer Research. S.K. also acknowledges funding from the Biotechnology and Biological Sciences Research Council (BB/M001873/1) and the Conrad N. Hilton Foundation.

10.1126/science.abh3188


EPIGENETICS

# The push and pull of DNA methylation

## The control of DNA methylation guides cell-fate decisions in development and disease

*By* **Tianpeng Gu** *and* **Margaret A. Goodell**

DNA methylation is an important covalent modification of mammalian genomic DNA that represses transcription. Genomic DNA is mostly maintained with high amounts of methylation, but some regions such as CpG islands are nearly perpetually unmethylated. Recently, large valleys or canyons of unmethylated DNA were discovered throughout the mammalian genome (*1*, *2*). Most are associated with conserved developmental regulators, such as homeobox genes, and are thought to be actively regulated. The size of these canyons is maintained by a push and pull interplay between DNA methyltransferases (DNMTs) and ten-eleven translocation (TET) dioxygenases that oxidize 5-methylcytosine, which leads to demethylation (*2*). On page 146 of this issue, Dixon *et al.* (*3*) identify QSER1 (glutamine and serine–rich protein 1) as part of the "push" protection mechanism that restricts DNA methylation. They show that QSER1 cooperates with TET1 by antagonizing chromatin binding of DNMT3A and DNMT3B, thus helping to retain the developmental potential of stem cells.

Chromatin binding of DNMT3A and DNMT3B, and hence their activity in establishing de novo DNA methylation, is directed by multiple factors, including nucleosomes (*4*), histone modifications (*5*, *6*), and specific interacting partners. For example, DNMT3A is recruited to intergenic regions through dimethylated lysine 36 of histone 3 (H3K36me2), whereas DNMT3B shows enhanced binding to gene bodies of actively transcribed genes in a trimethylated H3K36 (H3K36me3)–dependent manner. Although the mechanism is still unclear, the long DNMT3A1 iso-


Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA.
Email: tianpeng.gu@bcm.edu; goodell@bcm.edu


GRAPHIC: KELLIE HOLOSKI/*SCIENCE*

# Epigenetic nucleotides enhance therapy

Skirmantas Kriaucionis

*Science* **372** (6538), 127-128.
DOI: 10.1126/science.abh3188