A synthetic lethal approach targeting mutant isocitrate dehydrogenase in acute myeloid leukemia

Amit Verma & Ulrich Steidl

Pathogenic mutations of the genes encoding isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) occur in people with acute myeloid leukemia or other tumors. A new study identifies a dependence of *IDH*-mutated cells on the anti-apoptosis regulator BCL-2 and indicates a 'synthetic lethal' strategy for the treatment of leukemias.

Isocitrate dehydrogenase (IDH) 1 and 2 catalyze the conversion of isocitrate to α-ketoglutarate. Point mutations in IDH1 (R132 or R140) and IDH2 (R172) occur in a variety of cancers, including myeloid malignancies such as myeloproliferative neoplasms, myelodysplastic syndromes, and acute myeloid leukemias (AMLs)[1,2]. Mutations in IDH1 or IDH2 lead to a neomorphic function, resulting in the production of the oncometabolite 2-hydroxyglutarate (2-HG)[3,4]. 2-HG contributes to leukemia pathogenesis in part by inhibiting 2-oxoglutarate–dependent dioxygenases that are involved in histone and DNA methylation and hence modulating epigenetic marks[5]. Recent studies have furthermore suggested that mutations in IDH are founder mutations; that is, that they occur early in preleukemic stem cells during the multi-step transformation process of AML formation[6]. This makes them particularly promising therapeutic targets[7].

Recently, small-molecule inhibitors of mutant IDH2 have been developed and have shown encouraging preclinical and early phase clinical activity in IDH2-mutant AML[8]. Inhibitors of mutant IDH1 were shown to inhibit glioma cell growth and are currently in clinical testing[9]. Such direct targeting approaches rest on the assumption of oncogene addiction, i.e., the maintained essentiality of mutant IDH for the malignant phenotype.

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In this issue of *Nature Medicine*, Ravindra Majeti and co-workers[10] pursued an alternative approach that leverages the concept of synthetic lethality, in which a reliance on another pathway for survival by mutant cells can be exploited for therapy development.

Chan *et al.*[10] first carried out a functional RNAi screen in AML cell lines expressing either wild-type IDH1 or R132H-mutant IDH1 ($IDH1^{R132H}$) together with a green fluorescent protein marker. In this manner, they found two members of the *BCL2* family of anti-apoptotic genes, *BCL2L2* and *BCL2* (also known as *BCL-W* and *BCL-2*, respectively), to confer the strongest synthetic lethality; cell viability after shRNA targeting of these genes was reduced in the cells expressing mutant IDH1 compared to wild-type IDH–expressing AML cells, suggesting that mutant IDH increases dependency of AML cells on expression of these proteins (**Fig. 1**). Furthermore, the authors found that elevation of intracellular 2-HG by treatment of cells with its precursor octyl-(*R*)-2-HG confers increased sensitivity of AML cells with reduced levels of BCL-2 to apoptosis, confirming the dependency on BCL-2 for survival of cells in which 2-HG levels are increased[10] .

The authors focused their further studies on BCL-2 because of the clinical availability of a highly specific BCL-2 inhibitor, ABT-199 (ref. 11). AML cell lines expressing mutated versions of *IDH1* or *IDH2* or cell lines treated with octyl-(*R*)-2-HG (which increases intracellular levels of 2-HG) were found to be much more sensitive to pharmacological inhibition of BCL-2 by ABT-199 (that is, apoptosis was induced at lower drug concentrations)[10]. These results were confirmed in primary samples from 33 AML patients. Despite inter-individual variability, AML cells from patients with *IDH* mutations were significantly more sensitive to ABT-199 treatment *in vitro* than cells from AML patients with wild-type IDH[10].

Chan *et al.*[10] then transplanted primary AML cells into immunocompromised mice to assess the effects of BCL-2 inhibition *in vivo*. The engraftment of *IDH1*-mutated AML cells was significantly inhibited upon ABT-199 treatment when compared to leukemia cells with wild-type *IDH*. To assess effects on leukemia stem cell properties, the authors also transplanted the primary mutated *IDH1* grafts of leukemia cells into secondary recipients and found that ABT-199-treated cells engrafted at significantly lower levels than vehicle-treated mutant AML cells[10]. This suggests that ABT-199 treatment is able to confer synthetic lethal effects on putative leukemia stem cells with mutated *IDH1* as well as *IDH1*-mutated leukemic blasts.

Chan *et al.*[10] also investigated the mechanism by which ABT-199 confers synthetic lethality on AML cells with mutant *IDH*. BCL-2 normally inhibits the activity of proapoptotic proteins such as BAX and BAK, which induce apoptosis via permeabilization of the outer mitochondrial membrane (**Fig. 1**). Chan *et al.*[10] show that ABT-199 treatment of AML cells with mutant *IDH* or AML cells with experimentally elevated 2-HG levels (by treatment with the precursor octyl-(*R*)-2-HG) led to greater cytochrome *c* release from the mitochondria and greater mitochondrial membrane depolarization in *IDH*-mutant AML cells than in wild-type–*IDH* AML cells (**Fig. 1**). To pinpoint the subcellular and molecular target that mediates the synthetic lethal phenotype of mutant *IDH* and BCL-2 inhibition, Chan *et al.*[10] studied isolated mitochondria and individual components of the electron transport chain in AML cells and found that 2-HG inhibited cytochrome *c* oxidase (COX) (also known as complex IV). This link between 2-HG levels and COX activity is consistent with previous findings in other tissues[12,13]. Importantly, Chan *et al.*[10] found that COX activity was also decreased in primary human *IDH1*- and *IDH2*-mutant AML cell samples in comparison to wild-type–*IDH* cells. Finally, by using other pharmacological inhibitors they found that COX inhibition is indeed sufficient to sensitize AML cells to apoptosis upon ABT-199 treatment[10].

At the mechanistic level, the findings reported by Majeti and colleagues[10] raise the possibility that mutated *IDH* may also contribute to leukemia pathogenesis through deregulation of mitochondrial bioenergetics, beyond

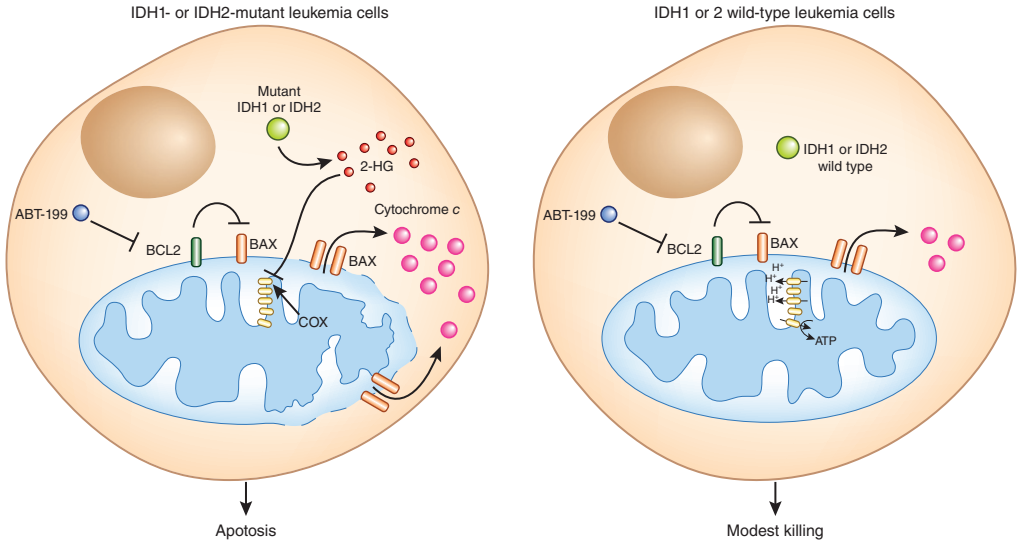

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Figure 1 Synthetic lethal mechanism of mutant *IDH* and BCL2 inhibition. Chan *et al.*[10] show that IDH1- or IDH2-mutant AML cells are sensitive to killing by treatment with the BCL-2 inhibitor ABT-199. BCL-2 normally inhibits the activity of apoptosis promoting proteins such as BAX, which increase mitochondrial membrane permeability. Mutant IDH1 or IDH2 produces 2-HG, which inhibits the mitochondrial membrane transport protein COX and makes the cell more sensitive to apoptosis upon BCL-2 inhibition (left). In wild-type cells, the mitochondrial electron transport chain is operative, conferring relative insensitivity to BCL-2 inhibition (right).

its well-appreciated role in epigenetic (dys) regulation. This idea is particularly intriguing in the context of recent reports of recurrent and functionally relevant mutations in mitochondrial genes, including genes encoding electron transport chain components in cancers[14,15], and thus it warrants further investigation.

In summary, the report by Majeti and colleagues[10] identifies the inhibition of BCL-2 as a synthetic-lethal approach to treating AML with mutant *IDH*. This is a finding of high translational importance. Although early clinical data on the first generation of IDH-mutant inhibitors in AML seem encouraging, it is unclear what percentage of patients will respond to such treatment and whether resistance will develop. Combinatorial approaches with BCL-2 inhibitors may offer a potential future strategy for enhancing IDH inhibitor treatment in AML patients. BCL-2 inhibition is a currently actively pursued strategy in AML and also in other cancers, and IDH mutation status may be one of the genetic factors that determines sensitivity to such treatment. The current study furthermore raises the possibility that, in patients with unmutated *IDH*, combination of ABT-199 with COX inhibitors may offer a novel strategy to enhance clinical efficacy and overcome potential resistance.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Parsons, D.W. *et al. Science* **321**, 1807–1812 (2008).
2. Mardis, E.R. *et al. N. Engl. J. Med.* **361**, 1058–1066 (2009).
3. Dang, L. *et al. Nature* **462**, 739–744 (2009).
4. Ward, P.S. *et al. Cancer Cell* **17**, 225–234 (2010).
5. Figueroa, M.E. *et al. Cancer Cell* **18**, 553–567 (2010).
6. Corces-Zimmerman, M.R., Hong, W.J., Weissman, I.L., Medeiros, B.C. & Majeti, R. *Proc. Natl. Acad. Sci. USA* **111**, 2548–2553 (2014).
7. Pandolfi, A., Barreyro, L. & Steidl, U. *Stem Cells Transl. Med.* **2**, 143–150 (2013).
8. Wang, F. *et al. Science* **340**, 622–626 (2013).
9. Rohle, D. *et al. Science* **340**, 626–630 (2013).
10. Chan, M. *et al. Nat. Med.* **21**, 178–184 (2015).
11. Souers, A.J. *et al. Nat. Med.* **19**, 202–208 (2013).
12. da Silva, C.G. *et al. Biochim. Biophys. Acta* **1586**, 81–91 (2002).
13. Latini, A. *et al. Mol. Genet. Metab.* **86**, 188–199 (2005).
14. Larman, T.C. *et al. Proc. Natl. Acad. Sci. USA* **109**, 14087–14091 (2012).
15. Silkjaer, T. *et al. Eur. J. Haematol.* **90**, 385–396 (2013).

Reducing peripheral serotonin turns up the heat in brown fat

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Obesity is a major risk factor for chronic disease. A new study in mice reveals that lowering levels of the signaling molecule serotonin outside of the brain reduces obesity and its complications by increasing brown adipose tissue (BAT) energy expenditure.

Accumulation of lipids in peripheral tissues, particularly the liver, is a driver of obesity-related complications[1]. One therapeutic strategy to prevent the onset of obesity-related diseases is to elevate fat oxidation in order to prevent excess lipid accumulation and to drive body weight reduction. However, there are currently no viable pharmacological agents that are able to do so safely. BAT has considerable potential as a candidate target tissue for obesity management. Its primary function is to protect against cold exposure through heat generation, and in doing so, it rapidly oxidizes fat and glucose. Although it was previously known to be important in mice and infant human physiology, it has only recently been shown to be present and functional in adult humans in response to acute cold exposure[2].

Although it was originally identified in the peripheral circulation for its vasoconstrictor actions, serotonin (5-hydroxytryptamine, (5-HT)) is better known for its central role in the regulation of mood, sleep, behavior and appetite (**Fig. 1**)[3]. Brain serotonin has an important role in energy balance, as it suppresses appetite and increases sympathetic

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