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Roles of IDH1/2 and TET2 mutations in myeloid disorders

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Abstract Mutations of the epigenetic enzymes isocitrate dehydrogenase (IDH) 1 and 2, and the methylcytosine dioxygenase 'ten–eleven translocation 2' (TET2), are common in human myeloid malignancies and drivers of these disorders but the underlying mechanisms remain obscure. This review examines mutant IDH1/2 and TET2 enzymes in the context of responses to DNA damage and their potential involvement in age-related genomic instability. The clinical relevance of these findings and their potential application in novel therapeutic strategies is also discussed.

Keywords IDH1 · TET2 · MDS · AML · DNA damage · DNA repair

Introduction

Recent advances in genomic sequencing have unveiled numerous novel somatic gene mutations in cancer cells, enabling characterization of unexpected functions for such genes. In myeloid malignancies such as myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), mutations in genes involved in epigenetics, including isocitrate dehydrogenases (IDH) 1 and 2, and methylcytosine dioxygenase 'ten–eleven translocation 2' (TET2), are not only common but also drivers of these diseases. Although mutated IDH1/2 are clearly established as oncogenes in myeloid cells, the mechanisms underlying their tumorigenic effects are not clear. Much evidence suggests that mutant IDH enzymes exert their effects via inhibition of TET2, but important clinical differences between *IDH1*-mutant and *TET2*-mutant hematopoietic disorders suggest that the oncogenic mechanisms of these mutated enzymes may differ. In particular, divergent effects of mutant IDH1/2 and TET2 on DNA damage repair mechanisms have been identified. In this review, we examine mutant IDH1/2 and TET2 in the context of responses to DNA damage and their potential involvement in genomic instability. We also discuss the clinical relevance of these findings and their potential application in novel therapeutic strategies.

Mutation of TET family enzymes

The TET family of dioxygenases is highly conserved and contains three members: TET1, -2 and -3. Somatic mutations of TET2 occur in various myeloid disorders, including chronic myelomonocytic leukemia (CMML) (~50 %), myeloid proliferative neoplasm (MPN) (~13 %), MDS (~25 %) and AML (~23 %) [1, 2]. TET2 is also mutated in B and T cell lymphoid malignancies, including angioimmunoblastic T cell lymphoma (AITL) [3]. More recently, *TET2* mutations have been detected in elderly individuals with clonal hematopoiesis of indeterminate potential, i.e.: individuals with clonal hematopoiesis without identified hematological disease. This indicates that TET2 mutations alone cannot drive leukemogenesis and that additional events probably contribute [4, 5]. Interestingly, *TET1* and *TET3* mutations are rare in human hematological diseases [6]. This predominance of TET2 alterations is not

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understood, particularly as conditional knockout (KO) of TET1, TET1/2 or TET2/3 in murine hematopoietic cells has profound biological consequences [7–9].

At the molecular level, TET enzymes catalyze the Fe(II)- and α-ketoglutarate (αKG)-dependent hydroxylation of 5-methylcytosine (5mC) residues in DNA to form 5-hydroxymethylcytosine (5hmC), which is involved in epigenetic regulation of gene expression [10]. 5hmC and other modifications trigger various mechanisms of DNA demethylation [11], and 5hmC inhibits the recruitment of methyl-DNA-binding transcriptional repressors to gene promoters. TET proteins also interact with O-linked β-*N*-acetylglucosamine (O-GlcNAc) transferase (OGT), which is responsible for the O-GlcNAcylation of histones that regulates gene transcription [12].

TET activity is essential for normal development in vivo. 5hmC is enriched in wild type (WT) murine embryonic stem (ES) cells, such that TET1/2/3 KO ES cells are dramatically depleted of 5hmC and exhibit poorly differentiated embryonic bodies and teratomas [13]. Promoter DNA in these embryos is hypermethylated, and genes involved in embryonic development and differentiation are deregulated. Similarly, in murine hematopoietic stem cells (HSCs) and progenitors, TET2 deficiency leads to impaired differentiation and increased self-renewal potential that drives a disease mimicking human CMML [14, 15]. However, as is true in humans, the development of AML in TET2-deficient mice requires additional alterations, such as a gain-of-function mutation of FLT3 [16]. Whether TET1/3 can compensate for loss of TET2 has yet to be addressed, but no increase in TET1/3 expression has been detected in TET2-deficient mice [14]. Moreover, human *TET2*-mutated AML cells show a decrease in 5hmC levels [17]. These data indicate that the mutations ultimately responsible for *TET2*-mutated diseases abrogate TET enzymatic functions, and also suggest that the epigenetic alterations resulting from TET abrogation may be involved in tumorigenic transformation.

Mutation of IDH1/2

IDH1/2 are homodimeric $NADP^+$-dependent enzymes that catalyze oxidative decarboxylation of isocitrate to produce the αKG, NADP, and CO_2 required for the Krebs cycle. In WT cells, IDH1 localizes to the cytoplasm and peroxisomes, whereas IDH2 resides in the mitochondrial matrix [18]. More than 80 dioxygenases require IDH-derived αKG to complete their enzymatic reactions. In particular, αKG regulates TET DNA hydroxylases and Jumonji C (JmjC) domain-containing histone demethylases (Fig. 1). αKG is abundant in ES cells and required for their pluripotent activity.

In 2008, sequencing of human tumor genomic DNA revealed missense mutations of IDH1/2 in glioma (~90 %), chondrosarcoma (~50 %), cholangiosarcoma (10–23 %), MPN (2.5 %), MDS (3.5 %) and AML (15–33 %) [1, 2, 18]. These mutations are heterozygous, mutually exclusive, and usually occur at one of three specific arginine residues: IDH1-R132, IDH2-R140 or IDH2-R172. Mutation of IDH2-R140 occurs exclusively in hematological disorders (usually myeloid) and not in solid tumors, whereas IDH2-R172 mutation is common in AITL [19].

Metabolite profiling studies later revealed that mutant IDH enzymes acquire a neomorphic activity that converts αKG to D-2-hydroxyglutarate (D2HG) in a reaction consuming NADPH [20]. This abnormal reaction is due to increased affinity of mutated IDH1/2 for αKG and decreased affinity for isocitrate. D2HG is an enigmatic metabolite in WT cells because it does not appear to function in any known metabolic process but is nevertheless detectable at low levels in both its D- and L-enantiometric forms. Both isoforms can be recycled back to αKG by D-2-hydroxyglutarate dehydrogenase (D2HGDH) [21] and L-2-hydroxyglutarate dehydrogenase (L2HGDH) [22]. D2HG was first identified in patients with D-2-hydroxyaciduria, which is caused by alterations to the D2HGDH gene or by IDH2-R140 mutations [23]. Levels of L2HG, but not D2HG, increase in cell cultured under hypoxia, and this elevation is associated with increased trimethyl H3K9. Trimethyl H3K9 is a marker of transcriptional repression, suggesting the involvement of L2HG in cell adaptation to hypoxia [24, 25]. However, the biological importance of the differing phenotypes caused by D2HG and L2HG has yet to be confirmed in vivo.

The structural similarity between αKG and D2HG has led to the hypothesis that D2HG is a competitive inhibitor of αKG-dependent reactions. L2HG has a greater capacity than D2HG to inhibit dioxygenases [26] but, to date, L2HG has not been implicated in oncogenesis, unlike D2HG. Dioxygenases vary in their vulnerability to inhibition by D2HG, ranging from the micromolar level for histone demethylases to the millimolar level for prolyl hydroxylase [26]. With respect to epigenetic enzymes, mutant IDH1/2 block TET1/2/3, resulting in decreased 5hmC and a hypermethylator phenotype in gliomas [27]. Excessive D2HG also inhibits JmjC histone demethylases, increasing levels of trimethylation on various histones. However, the physiological consequences of these changes to histone marking are unclear.

D2HG-mediated inhibition is not restricted to epigenetic enzymes. We found, using a conditional IDH1 R132 knock-in (KI) mouse model, that D2HG impairs collagen maturation and may influence HIF1α and responses to hypoxia in brain [28]. In vitro, Xu et al. demonstrated that D2HG can inhibit HIF1α degradation by blocking proline hydroxylation [29], but Koivunen et al. then showed that D2HG can also activate prolyl hydroxylase domain-containing protein 2,

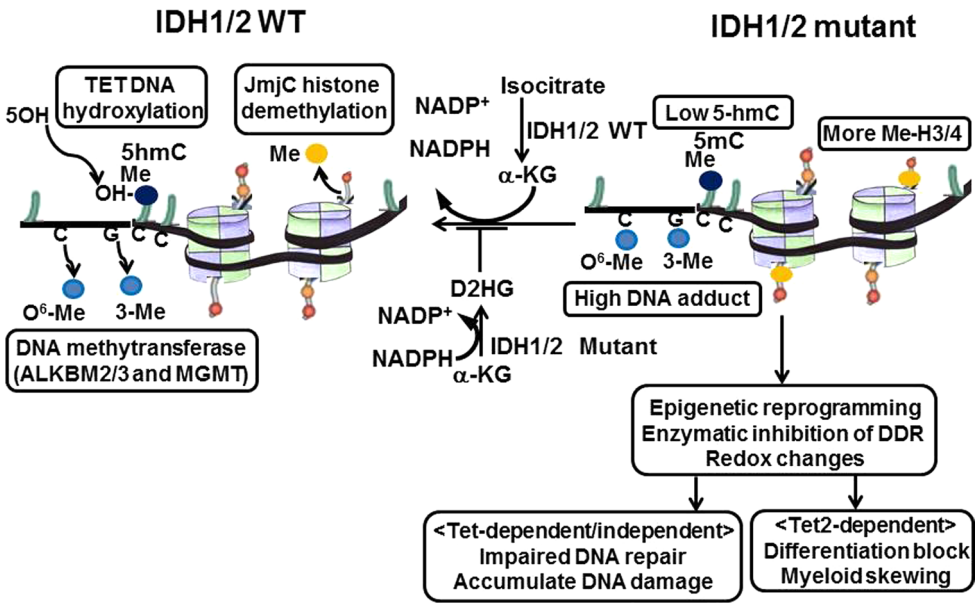

Fig. 1 Model of the effects of IDH1/2 mutations on DDR/DNA repair. The wild type IDH1/2 enzymes produce α-ketoglutarate (α-KG) and NAPDH. α-KG is required for the function of many enzymes, including DNA hydroxymethylases (TET family), histone demethylases (JmjC family) and DNA methyltransferases (ALKBH2/3 and MGMT). TET enzymes hydroxylate 5-methylcytosine (5mC) to produce 5-hydroxymethylcytosine (5hmC), while JmjC enzymes remove methyl groups (Me) from the lysine residues of histones H3/4. These reactions regulate the histone code and chromatin structure. If a single-strand DNA break (SSB) naturally occurs, the DNA repair enzymes ALKBH2/3 and MGMT act to maintain genomic integrity. MGMT converts mutagenic *O*-6-methylguanine to guanine whereas ALKBH2/3 remove the methyl groups from mutagenic 1-methyladenine and 3-methylcytosine and convert them to adenine and cytosine. All these α-KG-dependent reactions maintain the appropriate status of epigenetics and DNA repair in hematopoietic stem cells (HSCs). On the other hand, mutant IDH1/2 convert NADPH and α-KG and to $NADP^+$ and D-hydroxyglutarate (D2HG). Because D2HG competitively inhibits α-KG-dependent reactions, mutant IDH1/2 enzymes heighten DNA damage (particularly DNA adducts) by not only disrupting epigenetic regulators involved in DDR/DNA repair but also by elevating oxidative stress due to increased $NADP^+$ production. The combined effects of all these changes lead to a block in myeloid differentiation and accumulation of DNA damage in HSC/myeloid progenitors that is oncogenic

which is encoded by Egln1 and decreases HIF1α levels [30]. In vivo, IDH1-R132-KI embryos show heightened HIF1α in the brain but no changes in the hematopoietic system [28, 31], suggesting that effects of D2HG on HIF1α are likely cell context-dependent. Lastly, in AML cell lines overexpressing IDH1-R132H, D2HG inhibited Cox IV and mitochondrial respiration in a manner dependent on Bcl2 [32].

IDH1/2 mutations may also have consequences that are independent of D2HG. In contrast to WT IDH1/2, which produce NADPH, mutated IDH1/2 produce $NADP^+$, which drives an increase in reactive oxygen species (ROS) that may alter hematopoietic differentiation or promote tumor transformation. In addition, because WT IDH1/2 activities are crucial for metabolism, IDH1/2 mutations disrupt normal Krebs cycle functioning and may alter the synthesis of amino acids and fatty acids [18].

TET2 and IDH1/2 mutations in hematological disorders

IDH1/2 and TET2 mutations affect epigenetic regulation and tend to be early events in leukemogenesis [33, 34]. TET2- or IDH-mutated hematopoietic cells display an overlapping DNA hypermethylation signature [35] that is associated with decreased 5hmC [17]. While IDH1/2 mutations are linked to lower levels of 5hmC than TET2 mutations, this difference is explained by the fact that some *TET2*-mutated patients are haploinsufficient for this enzyme. In MDS and AML patients, the driver *IDH1/2* and *TET2* mutations coexist with similar "secondary" mutations, including alterations affecting *FLT3*, *NPM1* and *RAS* [33, 35–37]. Likewise, TET2 KO mice and IDH1-R132-KI mice show parallel phenotypes, including global 5hmC reduction, altered DNA methylation, blocked HSC/progenitor differentiation, myeloid skewing, and myeloid disorders [14, 15, 31].

Despite the above, substantial differences exist between *IDH1/2*-mutated and *TET2*-mutated hematological disorders. *TET2* mutation is more common in MDS/MPN than in AML, whereas *IDH1/2* mutations are more frequent in AML than MDS/MPN [1, 2]. When present in MDS, *IDH1* mutation is associated with shorter leukemia-free survival and decreased overall survival, whereas these impacts are not found in *TET2*-mutated MDS [38, 39]. Furthermore,

unlike *TET2* mutations, *IDH1/2* mutations are quite rare in clonal hematopoiesis of indeterminate potential. *IDH1/2* mutation frequency is higher in de novo AML than in chronic myeloid neoplasms [40], whereas the greatest incidence (50 %) of *TET2* mutations occurs in CMML, in agreement with the myelomonocytic proliferation in TET2 KO mice. Thus, IDH1/2 mutations appear to have higher oncogenic potential than *TET2* mutations and are associated with more aggressive diseases [1, 2].

A key observation has been that *IDH1/2* and *TET2* mutations are mutually exclusive in myeloid neoplasms. Since D2HG inhibits TET2 enzymatic activity, it has been postulated that the effects of mutated IDH in MDS/AML are due solely to D2HG-mediated TET2 inhibition and the resulting alterations to DNA methylation in HSCs and myeloid progenitors [18]. This hypothesis is in line with the strong inhibition of TET2 activity in cells of patients with IDH-mutated AML [35]. However, AITL, which is one of the most frequent T cell lymphomas, stands in contrast to the preceding. These lymphomas often exhibit *TET2* mutations (50 %) or *IDH2* mutations (20–30 %). Surprisingly, these two mutations are not mutually exclusive and coexist in the same tumor in 60–100 % of *IDH2*-mutated AITL patients [41, 42]. Thus, *TET2* mutation and *IDH1/2* mutation have at least some differing tumorigenic effects, even if they share a communal oncogenic mechanism.

The prognostic impact of IDH1/2 mutations in AML is unclear, and could depend on the specific IDH mutational hotspot and on associated secondary mutations. However, in some studies, AML patients with both *IDH1/2* mutation and *NPM1* mutation have a better prognosis after treatment with the daunorubicin than do TET2-mutated AML patients treated with this drug [43]. As daunorubicin is a DNA damage-targeting drug, this result could be explained by an effect of mutated IDH1/2 on the repair of damaged DNA. These findings are of great interest, and investigation of this possibility in multiple and larger patient cohorts would help to clarify this point.

Repair of damaged DNA in HSCs

HSCs continuously self-renew throughout life, whereas hematopoietic progenitors are relatively short-lived and differentiate into mature blood cells. It is widely believed that HSCs must possess unique cyto- and geno-protective mechanisms to ensure their homeostasis and long-term functionality. Indeed, WT murine HSCs are extremely quiescent, maintaining low levels of glycolytic metabolism and ROS. This quiescence is an attempt to minimize the chance of introducing genomic errors that could be caused by endogenous stressors such as cellular respiration and DNA replication.

In response to DNA damage, normal cells activate the DNA damage response (DDR), which is mediated by intracellular cell signaling that implement cell-cycle checkpoint controls and repair DNA breaks. A cell first activates the sensor kinases, such as ATM and ATR, which recognize DNA lesions and recruit the appropriate effectors to either arrest the cell and repair the damage or induce apoptosis. For double-strand DNA breaks (DSBs), two major DNA repair pathways are utilized: non-homologous end-joining (NHEJ) and homology-directed repair (HDR). There is a plethora of proteins, including ATM, Chk2 and p53, that play critical roles in these processes. Most single-strand breaks (SSBs) are dealt with by base excision repair (BER), nucleotide excision repair (NER), or DNA mismatch repair (MMR). Defects in many of these elements are associated with tumorigenesis [44].

HSCs are unique in that most DNA damage and repair signals are attenuated in these cells [45], leaving active only the rapid but error-prone NHEJ mechanism [46]. Next generation whole-genome sequencing of HSC cDNAs from normal humans of various ages has confirmed that HSCs serve as a reservoir for the accumulated DNA damage underlying hematopoietic disorders such as AML and MDS [33, 47]. Initial mutations of driver genes, including IDH1/2 and TET2, that occur in HSCs can clonally progress as preleukemic events, eventually leading to AML [1, 48].

In mice, HSCs lacking components of DDR and DNA repair pathways have significant deficits. The γH2AX foci that indicate DNA damage and accumulate in HSCs of older WT mice are found in even greater numbers in HSCs of mice lacking DDR function [49–51]. Mutations in DNA damage and repair genes result in unrepaired DNA damage in murine HSCs that leads to accelerated decline of their self-renewal capacity with age.

In contrast to most of the DNA repair mechanisms described above, there are two types of methyltransferases, namely members of the Alkylation Repair Homolog (ALKBH) family and *O*-6-methylguanine-DNA methyltransferase (MGMT), that can repair alkylated DNA using chemical reversion processes that do not require a nucleotide template. ALKBH enzymes convert the mutagenic nucleotides 1-methyladenine and 3-methylcytosine to adenine and cytosine, respectively [44]. ALKBH enzymatic activity depends on αKG and so may be inhibited by the D2HG produced by mutant IDH1/2, allowing alkylated DNA damage to accumulate. Another example is the conversion of the naturally-occurring, mutagenic nucleotide *O*-6-methylguanine to guanine by MGMT [44]. Notably, the MGMT gene is epigenetically silenced in several cancers [6]. Temozolomide is an alkylating agent that converts guanine to *O*-6-methylguanine, an event that eventually precipitates the death of the tumor cells. Indeed, temozolomide treatment of AML patients whose tumor cells

show MGMT downregulation has shown clinical promise [52]. Thus, it is interesting to investigate levels of MGMT expression in *IDH1/2* and *TET2* mutated AML.

Since 2015, the roles of the IDH and TET family enzymes in DDR/DNA repair have been investigated. TET1 KO mice develop spontaneous B cell lymphomas, and gene set enrichment analysis of RNA-seq from TET1 KO LSK and MPP cells showed that DNA repair genes, particularly the BER genes Apex1, Lig1 and Exo1, were downregulated [7]. Accordingly, γH2AX foci were prominent in TET1 KO pro-B cells. Intriguingly, whole-exome sequencing of human TET1-deficient non-Hodgkin B cell lymphomas revealed an increase in missense mutations, suggesting that TET1 loss can heighten the mutational burden in this lymphoma, even it does not lead to myeloid malignancy [7].

In contrast to TET1 KO mice, TET2/3 DKO mice develop AML within 2 months [8]. Gene expression analysis demonstrated deregulation of HR- and NHEJ-related genes in GMP and $CD11b^{+}$ myeloid cells of these mutants but a much milder effect in LSK cells. Although *TET3* mutation is not clinically relevant in humans, these mouse studies emphasize that a decrease in 5hmC levels impinges in multiple ways on the repair of damaged DNA. Interestingly, there is so far no evidence of DDR alterations in TET2 KO mice or *TET2* mutated AML patients. It may be that *TET2* alterations alone are unable to impair DDR, and that additional anomalies, such as TET3 or TET1 mutations, are required to derail this response.

An effect of IDH1 mutation on DNA damage/repair was also demonstrated by Molenaar et al. using an HCT116 cell line expressing mutant IDH1-R132 [53]. In this system, decreased NADPH production due to the mutated IDH1 enzyme increased ROS, leading to DNA damage induced by oxidative stress (Fig. 1). Indeed, HCT116 cells expressing mutant IDH1-R132 showed increased radio-sensitivity that was reversed by AGI-5198, a specific inhibitor of mutant IDH1 enzymes [54]. Also, Wang et al. used U87MG and HT1080 cell lines overexpressing mutant IDH1 to show that D2HG inhibits the ALKBH family of DNA repair enzymes [55]. As ALKBH activities depend on αKG, these cell lines showed decreased DNA repair, accumulation of DNA damage, and sensitivity to alkylating agents such as methyl methanesulfonate (MMS) and *N*-methyl-*N*′-nitro-*N*-nitroguanidine (MNNG) (Fig. 1). Interestingly, although the above reports indicate that mutant IDH1-R132 reduces DNA repair capacity, their underlying mechanisms differ. It is possible that these differences, which remain under investigation, are cell context-dependent.

Clinical implications

The clinical relevance of defects in DDR/DNA repair in AML has been controversial because AML has a low mutational burden and key DDR/DNA repair genes are rarely mutated in this disease [6]. It may be relevant that AML arises primarily de novo and is characterized by a block in myeloid progenitor differentiation that leads to abnormal expansion. Slush et al. reported that clonal AML arises from a pool of preleukemic cells representing a heterogeneous group of subpopulations [36, 37]. Genomic diversity was observed only in non-clonally expanded populations and not in clonally expanded homogeneous populations. Thus, the bulk DNA sequencing strategy commonly used for TCGA analysis could be inappropriate in the AML context because the detection limit is too low to identify a non-clonal mutation even if it exists. The deep sequencing of genomic DNA isolated from non-clonally expanded single cells from patients with *TET2*- or *IDH1/2*-mutated AML should provide insight into the true effects of these mutations on genomic instability in AML.

The findings from mouse models that mutant IDH and TET enzymes do influence DDR/DNA repair and thus potentially transformation point towards new therapeutic strategies for IDH-mutated cancers. Several drugs that target DDR by exerting genotoxic stress have a long history of clinical use, including for AML. These agents kill tumor cells by alkylating DNA [carmustine (BCNU) and temozolomide], crosslinking DNA (cisplatin), or inhibiting DNA replication (gemcitabine, daunorubicin, fludarabine). Other drugs, currently in clinical trials, target specific DDR molecules, such as PARP (Olaparib), ATR (AZD6738), and MGMT (Lomeguatrib). A combination approach may also be of benefit. In preclinical models of AML, PARP inhibitors are most effective in killing AML cells in which the HDR repair mechanism is also impaired [56]. Thus, it will be interesting to investigate the sensitivity of IDH1/2- and TET2-mutant AML to PARP inhibitors. Other genes may also affect the efficacy of DDR targeting agents, and, due to the complex relationship between DDR and hematopoietic differentiation, mutant IDH1-expressing cells may harbor other targetable vulnerabilities. All these possibilities require investigation in large and independent patient cohorts.

Closing remarks

This review has examined mutant IDH1/2 and TET2 enzymes in the context of DDR and their potential involvement in tumorigenic genomic instability. The clinical relevance of these findings is becoming clear, and exciting new therapeutic strategies exploiting these observations should improve treatment options for patients with hematological disorders.

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