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Genome-Epigenome-Senescence: Is TET1 a caretaker of p53 injured lung cancer cells?

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Significance Statement

The study by Filipczak and colleagues identified the interplay between mutant p53 proteins and methylcytosine dioxygenase ten-eleven translocation 1 (TET1) in lung cancers. p53 transversion mutations were closely associated with high TET1 expression, which prevented genomic instability-associated cellular senescence. Depletion of TET1 was synergistic with classical anti-tumor drugs, such as cisplatin or doxorubicin, providing an attractive rationale for targeted therapies against TET1 combined with anti-tumor drugs in p53 mutant lung cancer patients.

Lung cancer is the leading cause of human cancer deaths worldwide. Despite recent advances in targeted therapy such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors for *EGFR* mutation-positive non-small cell lung cancer (NSCLC), a considerable number of patients experience disease recurrence and patients lacking such mutations are generally less sensitive to these targeted therapies. In addition, certain subsets of patients, such as those with *TP53* alterations, have a worse prognosis and are relatively resistant to chemotherapy and radiation. Therefore, the development of therapies including targeted and combination therapies are required to improve survival of NSCLC patients.

Previous studies have reported that members of the Ten-eleven Translocation (TET) methylcytosine dioxygenase family (TET1, TET2, and TET3) are anti-tumorigenic in a variety of cancers including hematological, gastric, colon, prostate, breast, and liver cancers. Specifically, decreased TET protein expression has been shown to confer cancer cells with high rates of transformation and proliferation. In hematological cancers, chromosomal aberrations and genetic mutations in the *TET2* gene are frequently found, which often results in decreased TET2 enzymatic activity. In contrast, TET mutations occur with relatively low frequency in solid tumors; in these cancers, mechanisms including overexpression of certain microRNAs and methylation of the TET1 promoter appear to play a larger role than *TET* mutations in the decreased expression level of TET1 (4, 5).

In this issue of *Cancer Research*, Filipczak and colleagues reported that TET1 is an oncogene in NSCLC, and suggest that its activation following loss of p53 may be exploited through targeted therapy-induced senescence (1). The authors examined TET1 expression in tumor-normal tissue pairs and tumor-normal lung-derived cell lines

with validation using TCGA RNA sequence data. Unlike previous studies, the authors found that NSCLCs (i.e., adenocarcinoma and squamous cell carcinoma) showed 2- to 90-fold *overexpression* of *TET1* at the mRNA and protein levels in 40% to 70% of the samples. Furthermore, they found that p53 with transversion mutations were unable to bind to the TET1 promoter and thus failed to repress TET1 transcription compared to wild-type p53. TET1 knockdown in p53 mutant cell lines effectively induced senescence through genomic instability manifested by DNA single- and double-strand breaks and the induction of p21. Importantly, the effect of TET1 knockdown was synergistic with cisplatin and doxorubicin. These data suggest that TET1 has a role in DNA repair and the prevention of genome instability in p53 mutant NSCLC cells and that targeting TET1 may provide a new treatment strategy for NSCLC patients with p53 inactive mutations.

The pattern of DNA methylation at cytosine bases in the genome is tightly linked to gene expression, and abnormalities in DNA methylation are observed in variety of diseases including cancer. The TET enzymes are 2-oxoglutarate and Fe (II)-dependent dioxygenases that catalyze the successive oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), to 5-formylcytosine (5fC), and to 5-carboxylcytosine (5caC) (2). TET1 and TET3 have a CXXC domain at their amino terminus, which recognizes non-methylated CpG islands to recruit chromatin-modifying activities, whereas TET2 does not. In spite of these structural differences, there is partial functional redundancy among the family members (3). These 5mc oxidation products (i.e., 5hmC, 5fC, and 5caC) are demethylated and converted into unmodified cytosine via the passive or active demethylation pathway. In the passive pathway, the 5mc oxidation products are simply not generated during rapid DNA replication, resulting in increased unmodified cytosine levels. In the active demethylation pathway, the 5mC oxidation products are

processed by several families of proteins: the activation-induced by the TET family, deaminase/apolipoprotein B RNA-editing catalytic component (AID/APOBEC) family, and thymine-DNA glycosylase (TDG) and base excision repair (BER) family of proteins. Gene expression is then activated upon DNA demethylation.

TET has been reported to be involved in various biological contexts. For example, TET1-induced 5hmC within regulatory regions has been shown to regulate the differentiation of the adult human intestine by a gene-specific mechanism and 5hmC alterations contributed to disrupted gene expression in colon cancer (6). A recent study showed that spalt like transcription factor 4 (SALL4A) was preferentially associated with 5hmC and occupied enhancers in mouse ESCs by a largely TET1-dependent mechanism. SALL4A facilitated 5hmC oxidation by stabilizing TET2 associations that fine-tuned the expression profiles of developmental genes in mouse ESCs (7).

Recent studies have also revealed the involvement of TET and DNA demethylation in genomic instability and DNA damage repair. Tet1 knockout in mouse embryonic fibroblasts resulted in increased double-strand breaks (DSBs) and genomic instability (9). Furthermore, Tet1 knockout mice showed a higher sensitivity to X-ray exposure. The underlying mechanisms between TET depletion and genomic instability might be explained by the downregulation of DNA repair associated genes in Tet1 knockout cells. In addition, studies showed the direct involvement of 5hmC in response to DNA damage, which colocalized with γH2AX and p53-binding protein 1 (53BP1) (10).

In addition to catalytic-activity-dependent mechanisms, recent studies have shown that TET may function by a catalytic-activity-independent mechanism. For example, TET1 can modulate gene expression through its association with polycomb group proteins, the SIN3A histone deacetylase complex, and male absent on the first (MOF)

histone acetyltransferase (2). Indeed, catalytically dead TET mutants have biological function and rescue the phenotype of TET depletion (8). This evidence indicates the complexity of TET functions in different contexts. Interestingly, Filipczak and colleagues demonstrated that TET1 depletion in p53 mutated NSCLC cells appeared to induce DNA damage and cellular senescence by a system different from the aforementioned mechanisms (1). Although the authors saw no substantial differences in 5mC, 5hmC and other epigenetic marks such as histone H3 lysine 27 trimethylation (H3K27me3), which is catalyzed by polycomb proteins, and H3K4me2 were found by the depletion of TET1, it significantly reduced H3K9me2, which is a repressive chromatin mark. A mechanistic link between TET1 and H3K9me2 was not fully examined in the study, but they did report that the depletion of TET1 reduced cell growth and altered the transcriptome independent of TET1 catalytic activity, which would be a previously undescribed epigenetic mechanism affecting genes related to genomic instability, DNA damage repair, and senescence.

There are several open questions arising from this study. How does elevated TET1 prevent lung cancer cells from genomic instability-associated cellular senescence in the context of mutant p53? Is this function of TET1 lung cancer specific? Does mutant p53 also upregulate TET1 expression in other cancer types? Are the other TET family proteins, TET2 and TET3, also involved in the prevention of cellular aging in cancer cells? Because this study showed that growth arrest caused by TET1 depletion in p53 mutant NSCLC cells was not mediated by any of the canonical mechanisms, further investigation into additional modes of senescence is warranted.

In conclusion, this is the first study showing that the interplay between mutant p53 transcriptional regulation and TET1 expression in cancer cells affect DNA damage,

genome instability, and cellular senescence. The development of TET1 inhibitors is an active area of research, and this study provides a new paradigm whereby targeting TET1 in combination with traditional anti-cancer drugs in p53 mutant NSCLCs may be an effective strategy for NSCLC treatment.

References

1. Filipczak PT, Leng S, Tellez CS, Do KC, Grimes MJ, Thomas CL, et al. p53-suppressed oncogene TET1 prevents cellular aging in lung cancer. Cancer Res. 2019.
2. Wu X, Zhang Y. TET-mediated active DNA demethylation: mechanism, function and beyond. Nat Rev Genet. 2017;18:517-34.
3. Dai HQ, Wang BA, Yang L, Chen JJ, Zhu GC, Sun ML, et al. TET-mediated DNA demethylation controls gastrulation by regulating Lefty-Nodal signalling. Nature. 2016;538:528-32.
4. Rasmussen KD, Helin K. Role of TET enzymes in DNA methylation, development, and cancer. Genes Dev. 2016;30:733-50.
5. Ichimura N, Shinjo K, An B, Shimizu Y, Yamao K, Ohka F, et al. Aberrant TET1 Methylation Closely Associated with CpG Island Methylator Phenotype in Colorectal Cancer. Cancer prevention research (Philadelphia, Pa). 2015.
6. Chapman CG, Mariani CJ, Wu F, Meckel K, Butun F, Chuang A, et al. TET-catalyzed 5-hydroxymethylcytosine regulates gene expression in differentiating colonocytes and colon cancer. Scientific reports. 2015;5:17568.
7. Xiong J, Zhang Z, Chen J, Huang H, Xu Y, Ding X, et al. Cooperative Action between SALL4A and TET Proteins in Stepwise Oxidation of 5-Methylcytosine. Mol Cell. 2016;64:913-25.
8. Kaas GA, Zhong C, Eason DE, Ross DL, Vachhani RV, Ming GL, et al. TET1 controls CNS 5-methylcytosine hydroxylation, active DNA demethylation, gene transcription, and memory formation. Neuron. 2013;79:1086-93.
9. Zhong J, Li X, Cai W, Wang Y, Dong S, Yang J, et al. TET1 modulates H4K16 acetylation by controlling auto-acetylation of hMOF to affect gene regulation and DNA repair function. Nucleic Acids Res. 2017;45:672-84.
10. Kafer GR, Li X, Horii T, Suetake I, Tajima S, Hatada I, et al. 5-Hydroxymethylcytosine Marks Sites of DNA Damage and Promotes Genome Stability. Cell Rep. 2016;14:1283-92.