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The observations of abnormal epigenomes in cancer has motivated the pursuit of epigenome-targeted drugs for cancer therapy, but despite the clinical approval of various drugs, their cellular mechanisms of action are only partially understood. A new study demonstrates that DNA methyltransferase inhibitors (DNMTi) and histone deacetylase inhibitors (HDACi) induce widespread cryptic transcription from transposable elements (TEs) that may contribute to cancer immunogenicity.

Brocks, Schmidt, Daskalakis and colleagues first analysed the transcriptional consequences of DNMTi and HDACi on the epigenetically silenced death-associated protein kinase 1 (*DAPK1*) gene in various cancer cell lines. Rather than observing a simple upregulation of canonical *DAPK1* transcripts, they discovered new cryptic transcription from unannotated intronic transcription start sites (TSSs), which resulted in 5'-truncated open reading frames.

To explore treatment-induced transcription initiation on a genome-wide scale, they carried out cap analysis of gene expression (CAGE) in NCI-H1299 lung cancer cells following treatment with DNMTi, HDACi or a combination of the two. They found that all regimens induced extensive *de novo* transcription, primarily from intronic and intergenic TSSs that are currently unannotated, which they termed treatment-induced non-annotated TSSs (TINATs). There was partial overlap between the TINATs resulting from single-agent DNMTi or HDACi treatment (175 of the 365 and 646 respective TINATs were shared), and combined DNMTi and HDACi treatment synergized to activate 2,354 TINATs. Epigenomic profiling of DNA methylation and various histone marks confirmed the largely distinct but synergistic mechanisms of action: whereas HDACi treatment alone increased acetylation of various histone residues at TINATs, DNMTi treatment resulted in a classic signature of promoter activation (comprising CpG demethylation and accumulation of H3K4me3, H3K9ac and H3K27ac marks), and the occurrence of these active promoter signatures was increased by combination treatment.

As DNMTi and HDACi treatment converge to activate a subset of shared TINATs, the team searched for underlying DNA sequence features that might explain the targeting. They found that TINATs were strongly enriched for TE sequence, particularly the LTR12 family of solitary long

terminal repeat (LTR). Specifically, CAGE indicated that it is the cryptic promoter within LTR12 elements that becomes activated, and that the presence of GATA2 binding sites in these promoters is associated with TINAT expression, hence the GATA2 transcription factor is likely to be involved in TINAT activation.

What is the functional consequence of LTR12-driven TINAT expression? Sequence analyses revealed that ~30% of TINATs were spliced into protein-coding genes, with various predicted functional consequences including protein truncation (for example, loss of regulatory domains, binding sites or cellular localization sequences), or protein fusions in which spliced, out-of-frame TINAT sequences contribute novel, putatively antigenic protein sequences. Cellular translation inhibition assays provided support for active translation of 13 out of 43 TINAT-spliced transcripts tested, although further proteomic studies will be required to assess the extent and functional consequences of TINAT-derived proteins more globally.

Part of the original rationale for developing DNMTi and HDACi for cancer therapy was to reactivate the expression of particular tumour suppressor genes that are selectively silenced in cancer cells. This latest study — and additional recent demonstrations of DNMTi treatment generating TE-derived double-stranded RNAs and a cytotoxic interferon response — highlight the wider transcriptional consequences of epigenome-targeted therapies, which is perhaps unsurprising given the genome-scale activities of DNMT and HDAC enzymes. As TEs are largely silent in both normal and cancer cells, one further challenge will be deciphering the extent to which these wider TE-driven transcriptional effects contribute to the observed partial cancer-cell selectivity of DNMTi and HDACi, such as whether the induced transcriptional effects are greater in cancer cells, or whether the immunogenic consequences of TE transcription enhances the immune targeting of cancer-specific antigens.

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