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When DNA Repair Backfires – Trabectedin Induces DNA Breaks in Active Genes

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Abstract: Many anticancer drugs are ineffective in tumors that have functional DNA repair mechanisms. In contrast, trabectedin, a tetrahydroisoquinoline alkaloid marine natural product, stands out as it is more lethal to cancer cells with active DNA repair, particularly transcription-coupled nucleotide excision repair (TC-NER), making it an intriguing alternative to standard chemotherapeutic agents. To optimize trabectedin's use in precision oncology, it is essential to understand how its toxicity depends on TC-NER. In this study, we reveal that incomplete TC-NER of trabectedin-DNA adducts generates persistent single-strand breaks (SSBs). These adducts are found to obstruct the second of two sequential NER-mediated DNA incisions. By mapping the 3'-hydroxyl groups of SSBs resulting from the first NER incision at trabectedin-DNA adducts, we achieve genome-wide visualization of TC-NER. Our findings show that trabectedin-induced SSBs predominantly occur in the transcribed strands of active genes, accumulating near transcription start sites. This work provides new insights into how trabectedin can be leveraged for targeted cancer therapies and for studying TC-NER and transcription.

Keywords: DNA repair · Genomics · Precision oncology · Trabectedin

Vakil Takhaveev works as a postdoctoral researcher and lecturer at the ETH Zürich Laboratory of Toxicology. He develops novel DNA damage sequencing methods and studies the mechanisms of anticancer drug action, carcinogenesis, and aging. Vakil completed his PhD in systems biology of metabolism in 2020 at the University of Groningen, Netherlands, and within the Marie Skłodowska-Curie European training network MetaRNA. He graduated with honors from the program of bioengineering and bioinformatics at Lomonosov Moscow State University, Russia, in 2015, having done his thesis project in transcriptomics at Leiden University Medical Center, Netherlands.

1. Introduction

Many anticancer drugs work by damaging DNA and disrupting replication and transcription but are ineffective in cancers with high DNA repair activity. Trabectedin (ET743) is a tetrahydroisoquinoline alkaloid marine natural product that forms DNA adducts at the N^2-position of guanine (Fig. 1a).[1] Trabectedin is approved for the treatment of sarcoma and ovarian cancer. The drug is unique in being more potent in cells with active transcription-coupled nucleotide excision repair (TC-NER), whereas TC-NER-deficient cells show resistance (Fig. 1b). TC-NER-proficient cells accumulate DNA breaks after the drug exposure,[2] suggesting that these breaks, not the adducts, drive toxicity. The precise mechanisms of trabectedin's TC-NER-dependent toxicity remains unclear, limiting its potential in precision medicine.

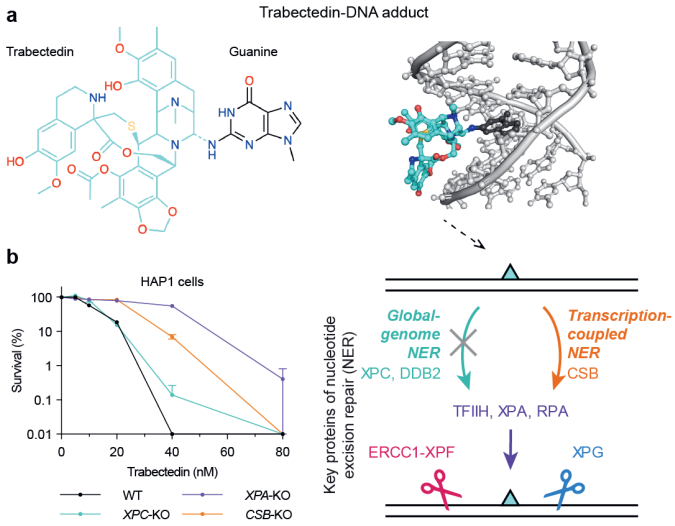

Fig. 1. **Trabectedin is more toxic to TC-NER-proficient *vs* deficient cells**. (**a**) Trabectedin-DNA adduct structure. (**b**) Left: survival of HAP1 wild-type (WT) and knock-out (KO) cells treated with trabectedin or DMSO for 2 h; colony counted after 8 days. Mean ±SEM, n=3. Right: schematic of two sub-pathways of NER with key proteins indicated.

2. Results

2.1 *Trabectedin Induces TC-NER-Dependent DNA Strand Breaks in G1 Cells*

We hypothesized that transcription-stalling trabectedin-DNA adducts trigger an abortive TC-NER reaction, leading to persistent

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SSBs. To test this, we employed high-throughput alkaline COMET chip assays.[3] In standard NER, acting for example on UV-induced DNA lesions, SSBs formed during strand incision exist only transiently and are quickly filled in by DNA repair synthesis, making them undetectable by COMET assays (Fig. 2a, *scenario i*). When DNA repair synthesis inhibitors (AraC/HU) are added to an NER reaction, these SSBs persist (Fig. 2a, *scenario ii*). With this method for break detection, we tested our hypothesis (Fig. 2a, *scenario iii*). To minimize background noise from replication-associated breaks, we synchronized cells in G1. Cells treated with trabectedin showed a 5-fold increase in SSBs within 4 hours (Fig. 2b-c). Similar levels of breaks were observed in XPC- and DDB2-deficient cells, ruling out global genome (GG)-NER involvement (Fig. 2c, see Fig. 1b, right for key proteins of NER). In contrast, breaks were absent in CSB- and XPA-deficient cells, confirming TC-NER dependency (Fig. 2c). These findings demonstrate that trabectedin induces DNA breaks *via* TC-NER.

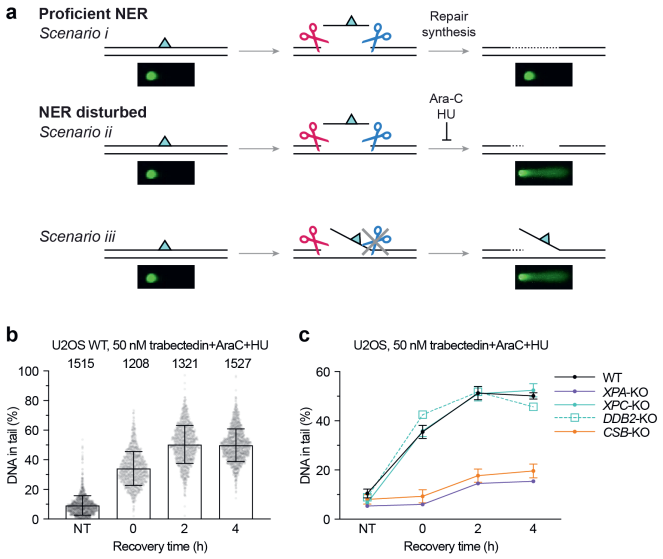

Fig. 2. **Trabectedin induces TC-NER-dependent DNA strand breaks in G1 cells**. (**a**) Scheme for assessing NER incision activity following DNA damage by alkaline COMET chip assays. (**b**) U2OS WT cells were arrested in G1 with palbociclib (1 µM, 24 h), treated with trabectedin (50 nM, 2 h) and allowed to recover for up to 4 h with repair synthesis inhibitors (1 mM HU, 10 µM AraC). ssDNA breaks were analyzed by alkaline COMET chip assays. Dot: DNA in tail (%) of a comet. Box: mean. The number of comets is above each box. Error bar: SD. (**c**) Summary of COMET chip experiments. Mean ±SEM of n=4 (WT, XPC-KO), n=3 (CSB-KO). n=1 (DDB2-KO and XPA-KO). Input data: mean values of individual experiments (boxes in b).

2.2 *Trabectedin-Induced DNA Breaks Depend on the Catalytic Activity of XPF but not that of XPG*

In NER, the XPF-ERCC1 complex makes the 5' incision before the XPG endonuclease makes the 3' incision (Fig. 1b, right).[4] We hypothesized that XPF activity, but not XPG activity, is required for trabectedin-induced break formation. Trabectedin-induced cytotoxicity depended on the catalytic activity of XPF, as ERCC1-KO and XPF-D687A cells were resistant to trabectedin (Fig. 3a) and showed no breaks after treatment, unlike WT cells (Fig. 3b). XPG-KO cells were resistant to trabectedin, but XPG-E791A cells, lacking catalytic activity, were sensitive, indicating that XPG presence, not activity, is necessary for XPF incision (Fig. 3c). COMET assays confirmed similar break levels in WT and XPG-E791A cells (Fig. 3d), suggesting that trabectedin-DNA adducts block XPG incision, leaving a persistent SSB with a free 3'-OH upstream of the lesion. Thus, trabectedin-induced break formation and toxicity rely on XPF activity but not on XPG activity (Fig. 3e).

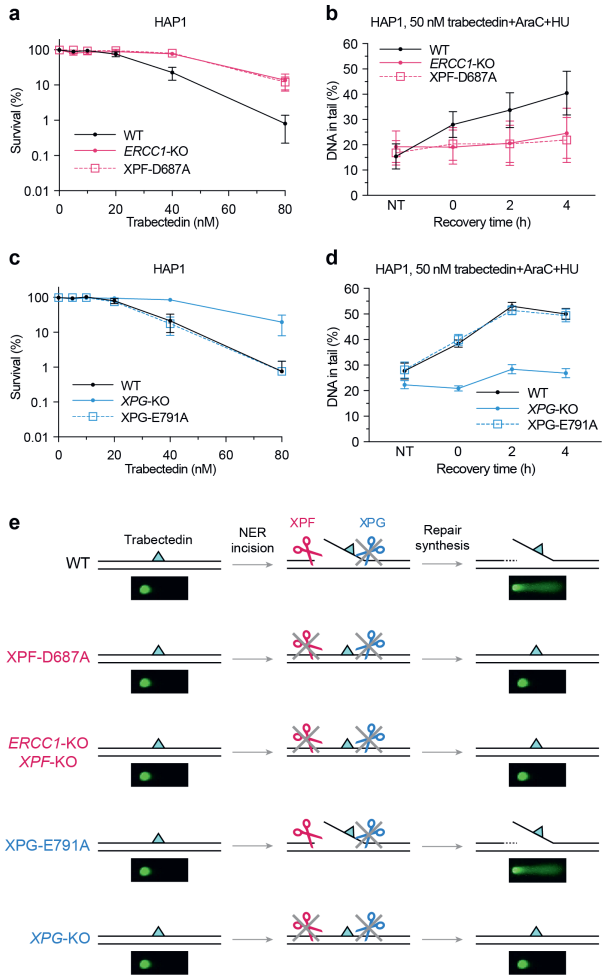

Fig. 3. **Trabectedin-induced DNA break formation and toxicity depend on the catalytic activity of XPF but not that of XPG**. (**a**,**c**) Indicated cell lines were treated with trabectedin for 2 h and colonies counted after 7 days. Mean ±SEM of n=5 (WT in a, XPF-D687A), n=3 (ERCC1-KO), n=4 (WT in c, XPG-KO, XPG-E791A). (**b**,**d**) Indicated cell lines were arrested in G1 with palbociclib and treated with trabectedin (50 nM, 2 h) and incubated for up to 4 h with repair synthesis inhibitors (0.5 mM HU, 5 µM AraC). ssDNA breaks were analyzed by alkaline COMET chip assays. Mean ±SEM of n=3 (b) and n=5 (d). (**e**) A schematic of NER incision activity on trabectedin-induced DNA adducts in the cell lines profiled by alkaline COMET chip assays.

2.3 *Trabectedin-Induced DNA Break Counts Correlate with Gene Expression Levels*

We hypothesized that it would be possible to map trabectedin-induced SSBs genome-wide and with single-nucleotide resolution. Using an upgraded GLOE-Seq method[5] called TRABI-Seq, we labeled and enriched DNA fragments from SSBs for sequencing (Fig. 4a). Thereby we mapped ERCC1-XPF-mediated trabectedin-induced breaks in TC-NER-proficient (WT, XPC-KO) and TC-NER-deficient (CSB-KO, XPA-KO) cells after trabectedin treatment. Trabectedin-induced breaks were abundant in WT and XPC-KO cells but absent in CSB-KO and XPA-KO cells (Fig. 4b). Breaks in WT cells correlated with transcriptionally active chromatin regions, DNase I hypersensitivity sites, and epigenetic marks of active promoters and enhancers (Fig. 4c).

We analyzed DNA break counts on transcribed (green) and non-transcribed (purple) strands relative to gene expression levels (Fig. 5a-b). The top 5% of expressed genes showed a 9-fold

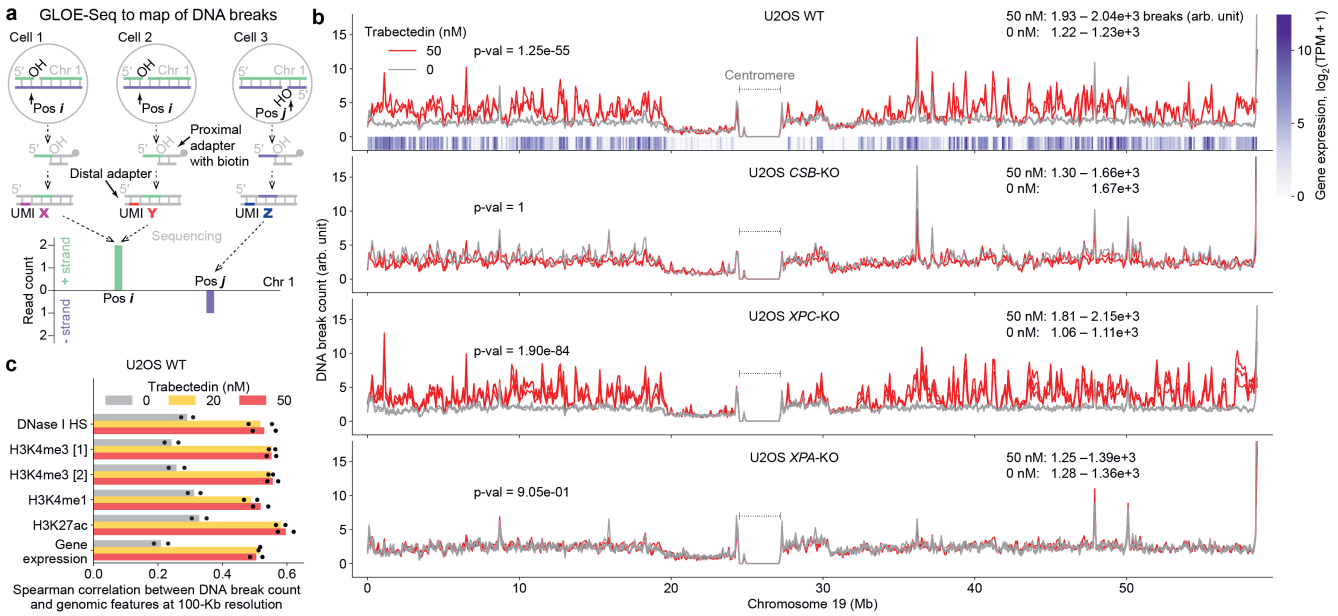

Fig. 4. **Trabectedin-induced DNA breaks are mapped genome-wide with upgraded GLOE-Seq**. (**a**) Principle of GLOE-Seq that maps DNA breaks in a genome-wide and strand-specific fashion and provides an estimate of the frequency of individual breaks in a population of cells. (**b**) DNA break count along chromosome 19 in 4 cell lines after 2 h exposure to trabectedin or vehicle (DMSO) and subsequent 2 h recovery. Solid lines: individual biological replicates, 2 for 50 and 0 nM drug in WT, 50 nM in CSB-KO; 1 for 0 nM in CSB-KO; 3 for 50 and 0 nM in XPC-KO and XPA-KO. P-value: Mann-Whitney U test with the one-sided alternative hypothesis that the trabectedin-treatment-related distribution is stochastically greater than the control distribution. TPM, transcripts per million transcripts. (**c**) Genome-wide correlation of DNA break count with the abundance of DNase I hypersensitivity (HS) sites (transcriptional activity), H3K4me3 (active gene promoters), H3K4me1 (active enhancers) and H3K27ac (active promoters and enhancer) as well as gene expression. Bars: mean, markers: biological replicates (n = 2) of break mapping. N = 28,513 genomic bins to compute the correlation.

higher break count on the transcribed strand compared to unexpressed genes, while the non-transcribed strand was unaffected by induced breaks (Fig. 5a). This pattern was absent in TC-NER-deficient CSB-KO cells, consistent with COMET chip results (Fig. 5b). The assay showed high reproducibility across biological replicates and a dose-dependent relationship.

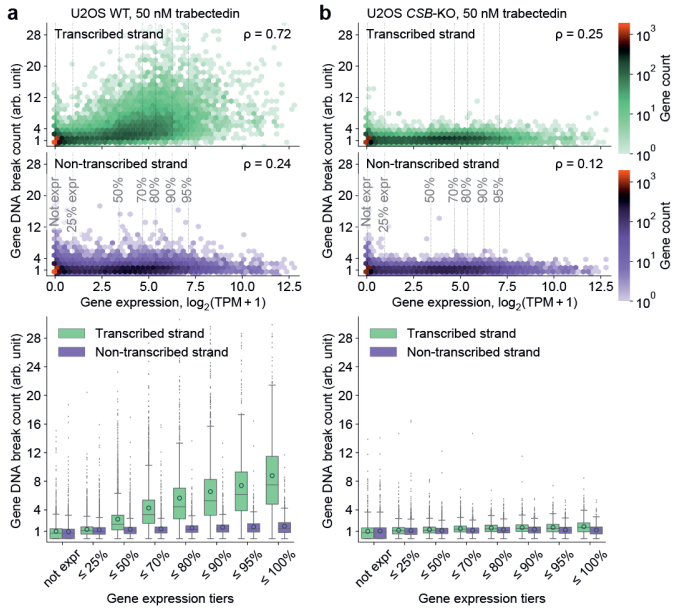

Fig. 5. **Trabectedin-induced DNA break counts correlate with gene expression levels**. DNA break count on each strand of protein-coding genes in U2OS WT (**a**) CSB-KO (**b**) after 2 h exposure to trabectedin and subsequent 2 h recovery versus gene expression level in unexposed U2OS WT. ρ: Spearman correlation coefficient (n = 16,740). TPM, transcripts per million transcripts.

2.4 *Divergent Transcription is Detected by Trabectedin-Induced DNA Breaks*

Trabectedin-induced DNA breaks gradually decreased throughout the gene body in TC-NER-proficient cells (Fig. 6a-b). Breaks peaked 1–2 kb downstream of the TSS, independent of gene length, likely due to RNA polymerase stalling at early trabectedin-DNA adducts, inhibiting transcription and TC-NER (Fig. 6c). On the non-transcribed strand, breaks peaked a few hundred nucleotides upstream of the gene body (Fig. 6a,c), consistent with divergent transcription from promoters.[6] These upstream breaks correlated with gene expression but were, on average, half as frequent as downstream breaks (Fig. 6d). Thus, due to the TC-NER basis of TRABI-Seq, this method can also report the genome-wide patterns of active transcription.

3. Conclusions

We uncovered that trabectedin blocks one of two incision reactions in TC-NER and used this finding to map TC-NER and gene expression activity on a genome-wide scale (Fig. 7).[7] Trabectedin is an approved drug to treat sarcoma and ovarian cancer. Our studies suggest that it is specifically effective for tumors with high DNA repair activity and so could provide additional use as an alternative to more traditional drugs such as cisplatin, for which NER has been shown to contribute to resistance. TRABI-Seq, the approach of break sequencing informed by the uncovered mechanism of trabectedin, provides opportunities not only to study TC-NER but also to profile tumor vulnerability by mapping TC-NER activity.

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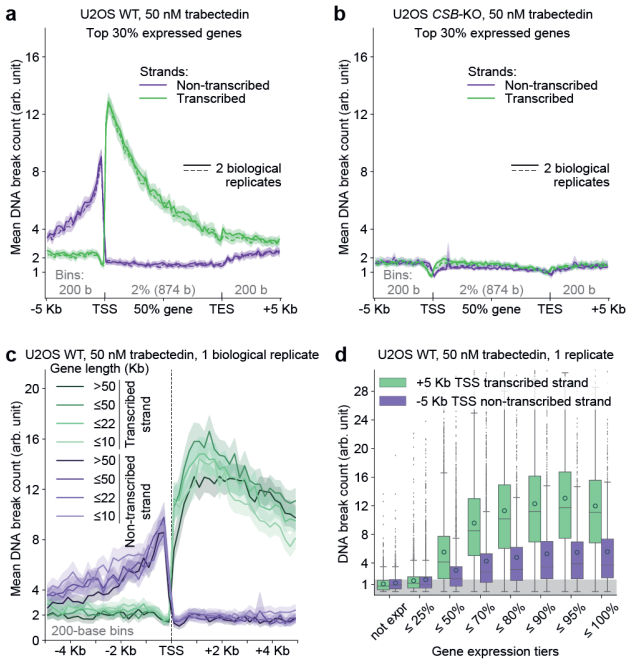

Fig. 6. TRABI-seq detects divergent transcription. Strand-specific profile of the mean DNA break count and its 95% confidence interval (c.i.) (shade) throughout the gene body and adjacent regions in U2OS WT (**a**) and CSB-KO; (**b**) after 2 h exposure to trabectedin and subsequent 2 h recovery. Solid, dashed and dotted curves means of different biological replicates. (**c**) Strand- and gene-length-specific profile of the mean DNA break count and its 95% c.i. (shade) in the ±5 kilobase (Kb) proximity of TSS. (**d**) DNA break count in two branches of divergent transcription versus gene expression.

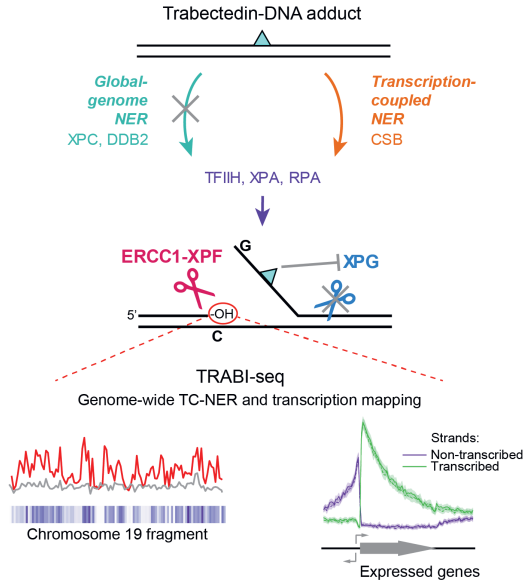

Fig. 7. Summary of the mechanism of trabectedin-induced TC-NER-mediated SSB formation and toxicity, and the development of TRABI-Seq.

Parts of this article, including parts of Figs. 1-7, have been published in open access form in 'Trabectedin derails transcription-coupled nucleotide excision repair to induce DNA breaks in highly transcribed genes', K. Son, V. Takhaveev, V. Mor, H. Yu, E. Dillier, N. Zilio, N. J. L. Püllen, D. Ivanov, H. D. Ulrich, S. J. Sturla, O. D. Schärer, *Nat. Commun.* **2024**, *15*, 1388, under CC BY 4.0 license. http://creativecommons.org/licenses/by/4.0/

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