TEX264 coordinates p97- and SPRTN-mediated resolution of topoisomerase 1-DNA adducts (TOP1ccs)

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Introduction

- DNA topoisomerase 1 (TOP1) resolves DNA supercoiling during replication and transcription but can become trapped on DNA, forming cytotoxic TOP1 cleavage complexes (TOP1ccs) that lead to genome instability.
- Persistent TOP1ccs are linked to diseases like neurodegeneration and cancer, while TOP1 inhibitors, such as camptothecins, exploit these lesions for cancer therapy.
- Resolving TOP1ccs requires upstream processing to remove bulky protein adducts, but the mechanisms remain unclear.
- This study investigates how TEX264, p97, and SPRTN coordinate TOP1cc repair, hypothesizing that TEX264 recruits p97 and SPRTN to enable proteolysis of TOP1ccs and subsequent DNA repair.

Methods

- **Mass spectrometry** was employed to identify TEX264 as a key partner interacting with TOP1.
- Using **siRNA (small interfering RNA) knockdowns**, the researchers evaluated TEX264's role in the repair of TOP1ccs by observing the effects of its depletion.
- **Microscopy techniques** were utilized to track the co-localization of TEX264, SPRTN, and p97 with TOP1ccs at DNA replication forks, providing visual confirmation of their interactions.
- DNA damage and repair efficiency were measured using **RADAR (Rapid Approach to DNA Adduct Recovery) and Comet assays**, which quantified TOP1cc accumulation and assessed the extent of DNA repair.
- Additionally, **protein interaction studies** confirmed that TEX264 acts as a bridge, linking p97 and SPRTN to TOP1ccs and facilitating their resolution.
- Functional analyses included experiments with TEX264 mutants to test specific domains required for interactions with TOP1 and SUMO-modified TOP1.
- These studies pinpointed the essential regions of TEX264 necessary for its activity in the repair of TOP1ccs.
- Chemical inhibitors targeting p97 ATPase activity were used to determine the importance of p97's remodeling function in the repair of TOP1ccs.
- Together, these studies provided a comprehensive understanding of how TEX264 and its partners contribute to the repair of TOP1ccs

Results

Figure 1a.

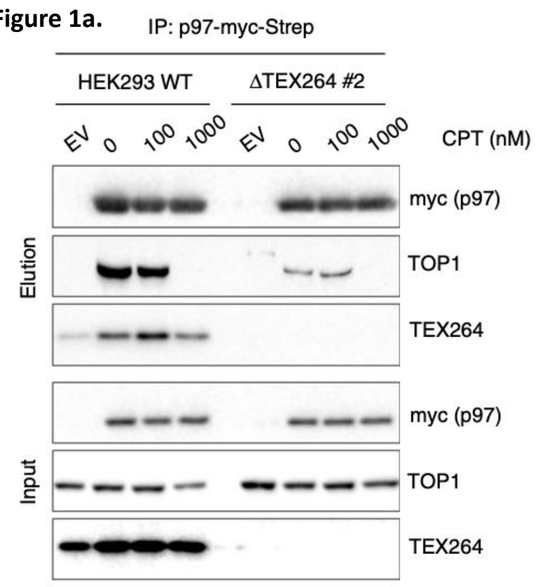

Figure 1b.

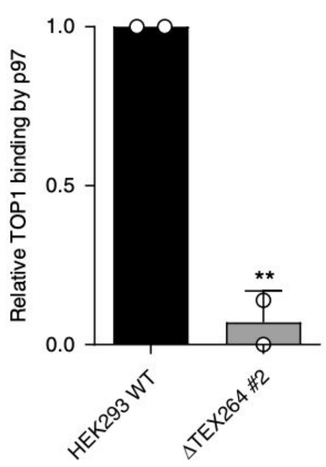

Figure 1. TEX264 bridges p97 to TOP1

- **Figure 1a**: Immunoblots of anti-Strep-tag immunoprecipitates prepared from wild-type (WT) or CRISPR-Cas9 TEX264 knockout (ΔTEX264) HEK293 cells expressing p97-Strep-Myc.
- **Figure 1b**: Quantification of two independent experiments (error bars represent mean ± SD; **P < 0.01; Student's t-test).
- TEX264 bridges the repair complex: TEX264 recruits p97 and SPRTN to stabilize and process TOP1 cleavage complexes (TOP1ccs).

Figure 2a.

Figure 2b.

Figure 2. TEX264 coordinates repair of TOP1ccs by action of p97 and SPRTN

- **Figure 2a**: RADAR assay to assess TOP1cc accumulation after short interfering (si)RNA-mediated depletion of p97. Treatment with 1 µM CPT for 1 hour was used as a positive control for TOP1cc induction. Double-stranded (ds)DNA is used as a loading control.
- **Figure 2b**: Quantification of A (error bars represent mean ± SEM; n = 2 for CPT (1 µM); n = 3 for siLuc, sip97 #1 and #2; *P < 0.05; ns, not significant; Student's t-test).
- The inhibition of p97 led to accumulation of TOP1ccs. As p97 unwinds the complex, the ATPase activity of p97 remodels TOP1ccs, leading to a decreased accumulation of TOP1ccs.

Figure 3a.

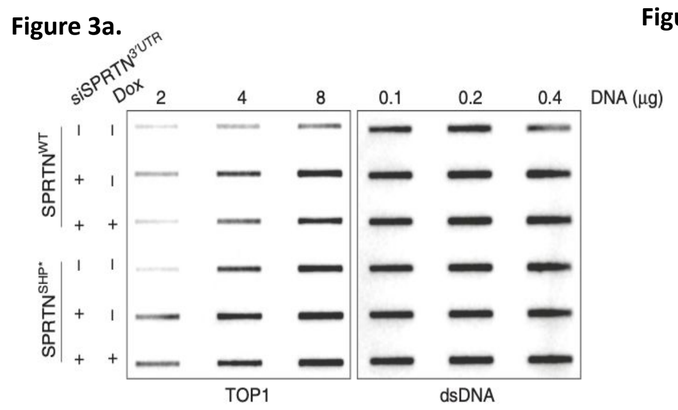

Figure 3b.

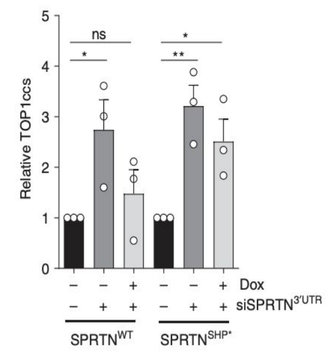

Figure 3. SPRTN and p97 proteolytically repair TOP1cc

- **Figure 3a**: TOP1cc detection by RADAR in SPRTN depletion/induction in doxycycline (Dox)-inducible HEK293 Flp-In T-REx cells.
- **Figure 3b**: Quantification of **3a** (error bars represent mean ± SEM; Student's t-test; n = 3; *P < 0.05; **P < 0.01; ns, not significant).
- SHP domain of SPRTN binds to p97: When SHP domain is inhibited, SPRTN was unable to recruit p97 leading to an increased accumulation of TOP1ccs
- SPRTN cleaves protein adducts: SPRTN proteolytically removes the bulky TOP1 protein, allowing downstream repair by TDP1.

Figure 4a.

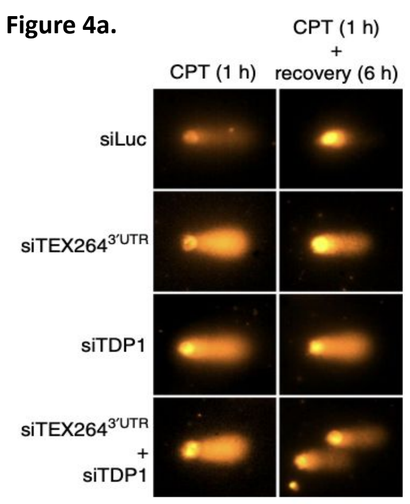

Figure 4b.

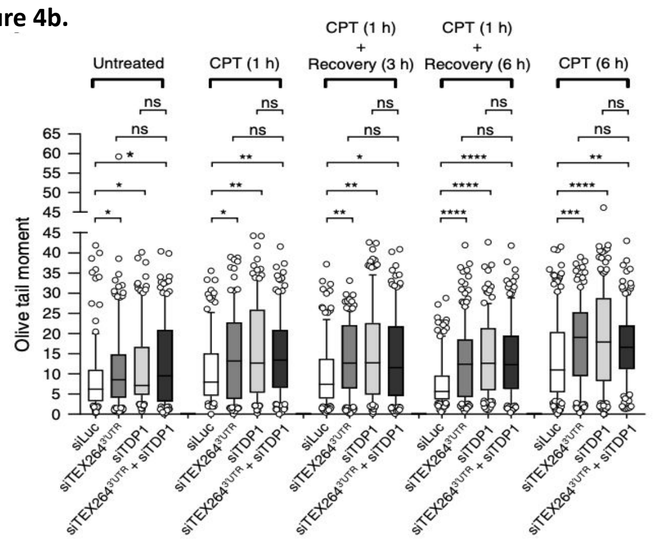

Figure 4. TEX264-deficiency accumulates TOP1ccs

- **Figure 4a:** The alkaline comet assay showed that TEX264-depleted cells had higher baseline DNA damage and delayed repair after camptothecin (CPT) treatment (100 nM) during 1- and 6-hour recovery periods.
- **Figure 4b:** Results from >100 cells per condition, consistent across two experiments, were analyzed using Kruskal–Wallis ANOVA with Benjamini–Hochberg corrections.
- TEX264-deficient cells are impaired: These cells accumulate TOP1ccs, experience replication stress, leading to increased DNA damage and thus exhibit heightened sensitivity to camptothecins.

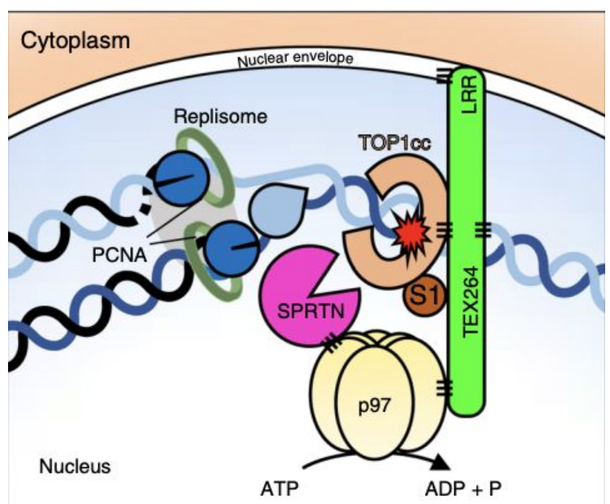

Figure 5.

Figure 5. Model of TOP1ccs repair by the p97-TEX264-SPRTN complex

- **Figure 5:** Proposed model: TEX264 is tethered at the nuclear periphery by its LRR. TEX264 binds to unmodified and SUMO1-modified TOP1 and counteracts TOP1cc accumulation by recruiting p97-SPRTN sub-complexes to TOP1ccs. S1 denotes SUMO1. Source data are available online.
- TEX264 interacts with SUMO1-modified TOP1: This interaction stabilizes the repair complex and promotes efficient resolution of TOP1ccs.

Clinical Implications

- This fundamental insight demonstrates the importance of upstream processing in DNA repair pathways.
- TEX264 could be inhibited to enhance sensitivity to TOP1 inhibitors in resistant cancers in the development of targeted cancer therapy.
- TEX264 levels might predict responses to TOP1-targeting drugs in biomarker development.

Conclusion

- TEX264, p97, and SPRTN form a coordinated repair complex for TOP1cc resolution.
- TEX264 is essential for genome stability and has potential as a therapeutic target.
- Potential future questions include whether TEX264 inhibitors can improve the efficacy of camptothecins, whether other DNA-protein crosslinks are processed by this pathway, and how TEX264 localization affects its activity at replication forks.