Direct Observation of an Deadly DNA Breakage Induced by Anticancer Drug Etoposide and Type II Topoisomerase

Type II topoisomerases resolve the entanglements and crossings of cellular DNA by introducing a transient gap on a DNA double helix, such that another piece of DNA can be transported through this opening. Several widely prescribed anticancer drugs increase the population of Type II topoisomerases-mediated DNA breakage, leading to massive DNA damage and death of cancer cells. Using synchrotron radiation, we determined the three-dimensional structure of a human Type II topoisomerases bound to DNA and to the anticancer drug etoposide, thus reveal structural details of drug-induced DNA breakage, and provide valuable information for developing safer and more effective anticancer drugs.

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Type II topoisomerases (TOP2s) are ubiquitous enzymes that play essential roles in cellular DNA transactions.[1] These two-fold symmetric enzymes transiently cleave a pair of opposing phosphodiester bonds four base pairs apart, generating a TOP2-DNA cleavage complex. Passage of a second DNA segment through this enzyme-bridged "DNA gate" and its resealing complete the topological change of the DNA. Failure to reseal can lead to cell death. Several potent anticancer drugs, such as etoposide, doxorubicin, and mitoxantrone, exploit this harmful aspect and promote the formation of cytotoxic DNA lesions by increasing the steady-state level of cleavage complexes.[2] Despite the extensive clinical use of these drugs, structural details of drug-stabilized cleavage complexes are still largely unresolved. We therefore determined the high resolution crystal structure of the DNA-binding and cleavage core of the human TOP2 β-isoform (residues 445-1201 (Fig. 1) in complex with DNA and a highly successful anticancer drug etoposide, representing the first observation of a TOP2 ternary cleavage complex stabilized by an anticancer drug.

This structure reveals the detailed interplays between protein, DNA and drug. The two etoposide molecules bind between the base pairs (+1/+4; –1/+5) immediately flanking the two cleaved scissile phosphates (Fig. 2(a)), thus physically block the TOP2-mediated resealing of DNA. Besides the intercalating aglycone core (ring A, B, C and D), the two protruding groups (the glycosidic group and E ring) of etoposide also mediate direct interactions with surrounding amino acid residues (Figs. 2(b) and 2(c)). The spatially constrained binding pocket of E ring (Fig. 3(a)) explains why modifications to this part usually compromise drug activity. In contrast, the relatively spacious binding pocket for glycosidic group

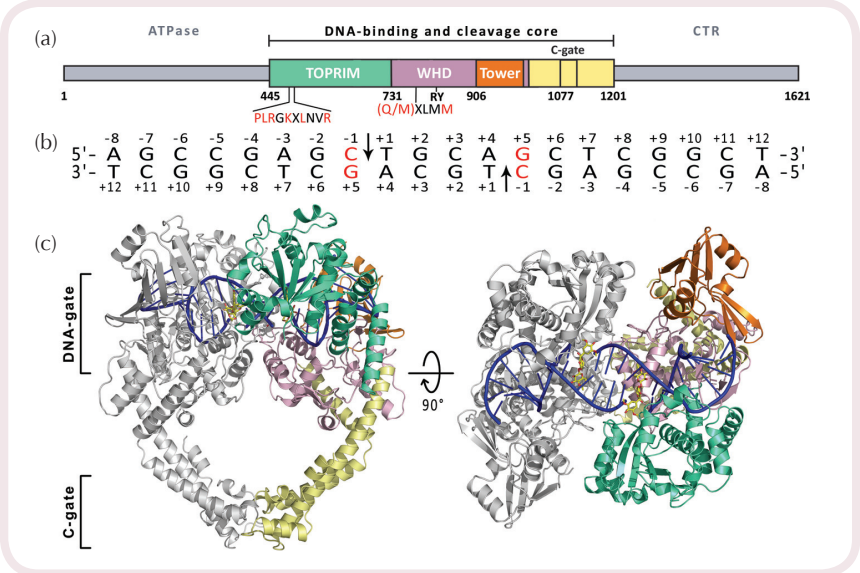

Fig. 1: Structure of the hTOP2 β^{core}-DNA cleavage complex stabilized by the anticancer drug etoposide. (a) Linear domain organization of hTOP2 β. (b) The palindromic DNA substrate used for crystallization. The –1/+5 base pairs shown in red highlight the nucleotide preference for this position. (c) Orthogonal views of the ternary cleavage complex.

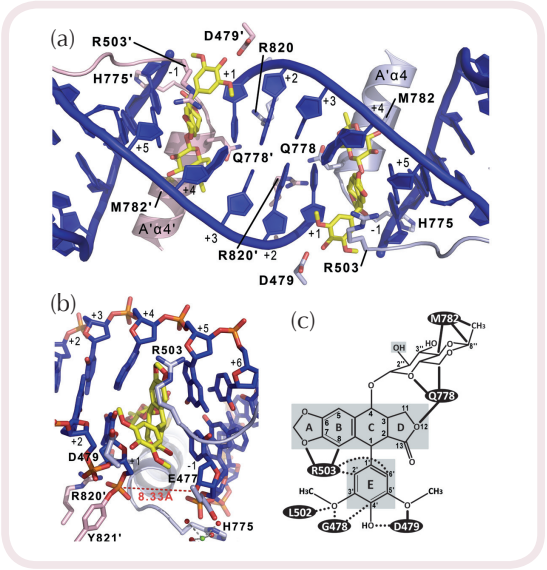

Fig. 2: Detailed views of the etoposide binding site(s). (a) Cartoon/stick representation shows the insertion of two etoposide molecules into two cleavage sites. (b) Close-up view of the etoposide binding site. The distance between the Tyr821'-linked scissile phosphate and the 3'-OH is indicated. (c) Chemical structure of etoposide. Atoms involve in drug-DNA interactions are shaded in gray.

(Fig. 3(b)) is more suitable than E ring for further modifications in developing better anticancer drugs. This structure also suggests that a cytosine at the –1 position is strongly favored at etoposide-stabilized DNA cleavage sites (Fig. 1(a)). We found that the +5 guanosine base not only stacks nicely with ring A and B of etoposide, but also anchors a major drug-contacting residue R503 by forming a H-bond (Fig. 3(c)). The apparent preference for having a guanosine at position +5 in turn specifies a cytosine at the –1 position. Many mutations in TOP2 may confer resistance to TOP2-targeting anticancer drugs and antibiotics.[3] We provided the structural basis of drug resistant by mapping reported mutation sites onto our structure, (Fig. 3(d)).

All vertebrates possess two highly similar yet functionally distinct TOP2 isoforms. The α-isoform is particularly important for DNA replication and is usually present at high levels in fast growing cancer cells, whereas the β-isoform is mainly involved in transcription-related processes.[4] Although the inhibition of both TOP2 isoforms contributes to the drug-induced death of cancer cells, targeting of the β-isoform has been implicated in deleterious therapy-related secondary malignancies.[5] This structure provides an opportunity to design isoform-specific TOP2-targeting agent. While most drug-contacting residues are conserved between isoforms, the reported structure further reveals that a key drug-interacting residue Q778 is replaced with methionine (M762) in the α-isoform. Such a change in residue polarity may be exploitable in developing new isoform-specific anticancer drugs with reduced side effects.

Beamline SPring-8 BL12B2 PX end station

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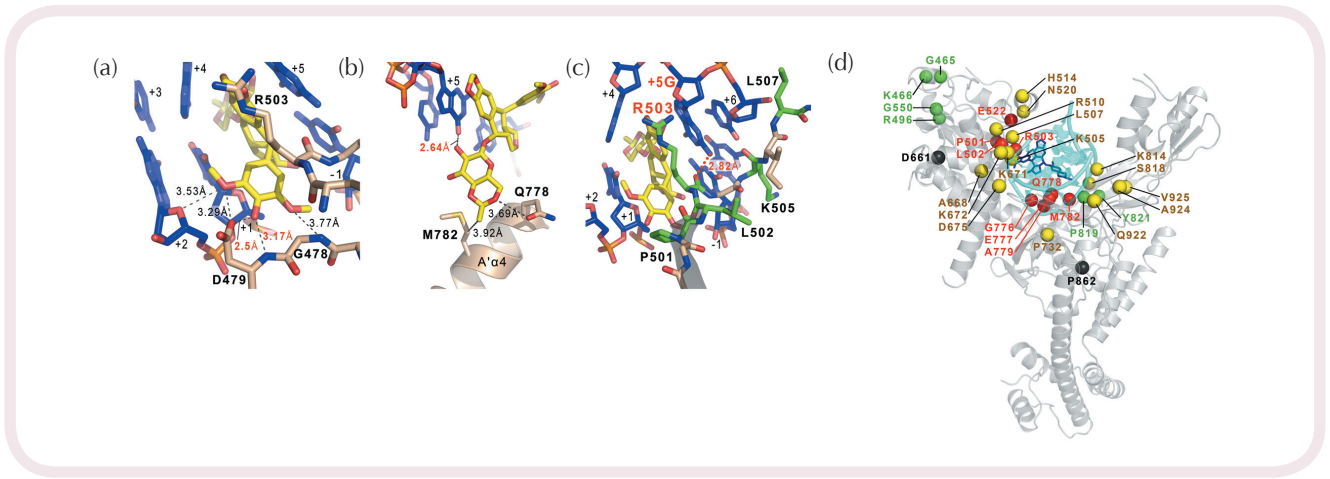

Fig. 3: Interactions observed in etoposide binding site suggest structure-activity relationships of etoposide derivatives, the sequence preference for drug-stabilized cleavage site, and the molecular basis of drug resistance. (a) and (b) Interactions mediated by the C1 (E-ring) and C4 substituent (glycosidic group) of etoposide. (c) The conserved PLRGKXL segment plays a key role in etoposide-binding by harboring R503. The red dashed line highlights a key hydrogen bond between the +5 guanine base and the main chain carbonyl of R503. (d) Spatial locations of the drug-resistant mutation sites reported for prokaryotic and eukaryotic TOP2s. Residues involved in drug-binding, DNA-binding, and catalytic functions are shown as red, yellow, and green spheres, respectively.