Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide

Type II topoisomerases (TOP2s) are ubiquitous enzymes that play essential roles in cellular DNA transactions including replication, transcription, recombination, and chromosome condensation and segregation [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.

TOP2's DNA cleavage activity is a double-edged sword; failure to reseal the enzyme-mediated DNA break can lead to cell death. Several potent anticancer drugs, such as etoposide, doxorubicin, and mitoxantrone, exploit this harmful aspect of TOP2 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, however, the lack of three-dimensional structures of any drug-stabilized cleavage complexes has left the structural bases of drug actions and resistance largely unresolved. To these ends, we determined the high resolution (2.16 Å) crystal structure of the DNA-binding and cleavage core of the human TOP2β-isoform (residues 445–1201; designated hTOP2β^{core}) (Fig. 1) in complex with DNA and a highly successful anticancer drug etoposide [3]. Structural analysis was carried out using Taiwan beamline **BL12B2**.

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 stabilize the cleavage complex by physically blocking 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, further highlighting the enzyme's central role in stabilizing the bound drug (Fig. 2(b) and 2(c)). Consistent with the observed structure-activity relationships of etoposide, 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. 3(b)) suggests that it is more suitable than E ring for further modifications in developing more effective anticancer drugs. This structure also suggests a molecular basis for a cytosine at the –1 position being 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, it 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. It is known that mutations in TOP2 may confer resistance to TOP2-targeting anticancer drugs and antibiotics. By mapping reported mutation sites onto our structure, we provided the structural basis of drug resistant (Fig. 3(d)).

This structure also offers molecular codes useful for the design of isoform-specific TOP2-targeting agent. This aspect is extremely important because 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]. Therefore, it is desirable to develop the isoform-specific TOP2-targeting agents. The reported structure further reveals that, while most drug-contacting residues are conserved between isoforms, a key drug-interacting residue Q778 is replaced with methionine (M762) in

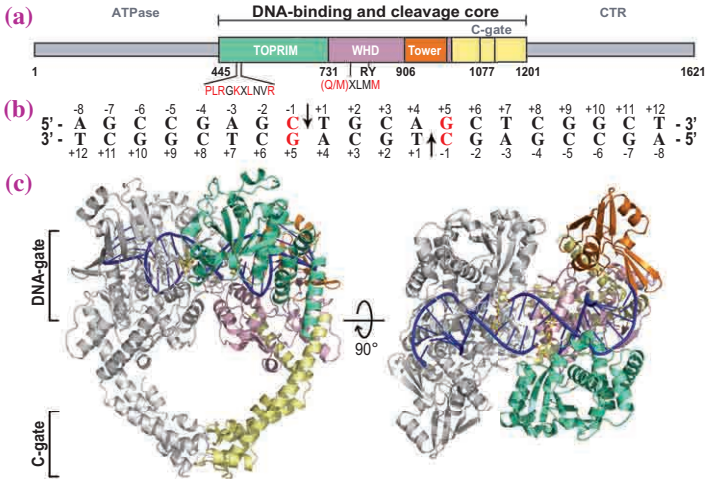

Fig. 1. Structure of the hTOP2β^{core}-DNA cleavage complex stabilized by the anticancer drug etoposide [3]. **(a)** Linear domain organization of hTOP2β. **(b)** The palindromic DNA substrate used for crystallization. The –1/+5 base pairs shown in red highlight the nucleotide preference for this position. **(c)** Orthogonal views of the ternary cleavage complex.

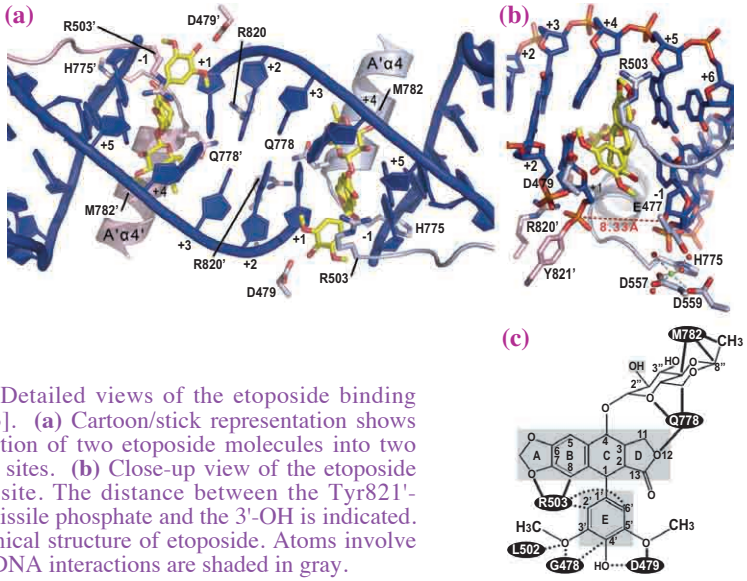

Fig. 2. Detailed views of the etoposide binding site(s) [3]. **(a)** Cartoon/stick representation shows the insertion of two etoposide molecules into two cleavage sites. **(b)** Close-up view of the etoposide binding site. The distance between the Tyr821'-linked scissile phosphate and the 3'-OH is indicated.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

the α-isoform. Such a change in residue polarity may be exploitable in developing new isoform-specific anticancer drugs with reduced side effects.

In summary, we represented the first observation of a TOP2 ternary cleavage complex stabilized by an anticancer drug. The interplays between protein, DNA and drug reveal structural details of drug-induced stabilization of a cleavage complex, and providing valuable information for developing a better anticancer drug.

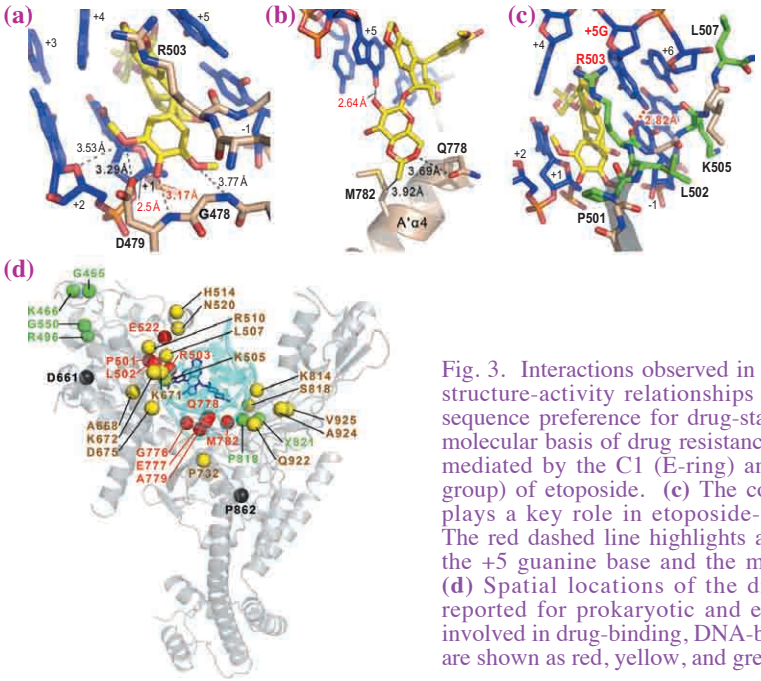

Fig. 3. Interactions observed in etoposide binding site suggest structure-activity relationships of etoposide derivatives, the sequence preference for drug-stabilized cleavage site, and the molecular basis of drug resistance [3]. **(a)** and **(b)** Interactions mediated by the C1 (E-ring) and C4 substituent (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.

Chyuan-Chuan Wu[a], Tsai-Kun Li[b] and Nei-Li Chan[a,*]

[a] Institute of Biochemistry and Molecular Biology, National Taiwan University, Taiwan
[b] Department and Graduate Institute of Microbiology, National Taiwan University, Taiwan

*E-mail: nlchan@ntu.edu.tw

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