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拓撲異構酶相關的抗癌症藥物之分子機制探討

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One of the key questions facing cancer pharmacology is the tumor specificity of the anticancer drugs. In fact, anticancer drugs are often referred as cytotoxic drugs due to their “non-specific” cytotoxic actions against both tumor and normal proliferating cells. Our studies, on the other hand, have started to reveal potential sources of antitumor specificity for DNA topoisomerase-directed anticancer drugs.

The key molecular event common to all topoisomerase-targeting anticancer drugs is the formation of a reaction intermediate, the topoisomerase-DNA covalent complex, which represents a new form of lethal protein-capped DNA damages. Initiation of subsequent cellular responses depends on the processing pathways that irreversibly converse cleavable complexes into exposed DNA breaks. In this application, we examined the requirements of cellular processing pathways prior to the initiation of cellular responses following formation of topoisomerase cleavable complexes with specific focus on interactions between enzyme-capped breaks, DNA tracking processes and proteasome.

Our preliminary results have suggested the involvement of transcription and 26S proteasome in the removal of DNA-linked enzyme. Based on these results, we propose that the transcription-dependent, proteasome-mediated degradation of topoisomerase represents as a new cellular pathway for processing TOP2 cleavable complexes into real stranded breaks, which can then serve as an initiation signal for various downstream pathways. Indeed, our results identified two independent processing pathways, transcription-initiated processing (TRIP) and replication-initiated processing, for DNA topoisomerase cleavable complex. The collision...
between RNA polymerase and topoisomerase cleavable complex (TRIP pathway) leads to proteasome-mediated removal of topoisomerase from topoisomerase-DNA adduct and exposes the DNA break. Distinct repair or checkpoint pathways are activated by replication collision (RIP) of topoisomerase-capped DNA breaks. In addition, we also explored the potential role(s) of MRE11/RAD50/NBS1 (MRN) complex in processing TOP2-mediated DNA damage.
Genetic damage is critically involved in carcinogenesis, neuron degeneration, aging as well as tumor cell killing by chemotherapeutic agents [1-7]. Among the forms of DNA damages, protein covalently linked to DNA represents a great genetic threat to genome integrity and cell viability [4-6, 8]. However, the cellular responses to protein-linked DNA lesions remain largely unclear. Interestingly, several lines of evidence indicate that telomere is a hidden DNA breaks capped protectively by telomere-associated proteins [8-14]. Exposure of protein-protected chromosome telomere ends (telomere dysfunction) triggers DNA damage pathways [8-15]. Notably, from studies of telomere dysfunction, cellular responses for uncapped DNA telomere ends reminisce DSB-induced pathways [8-14], arguing that the physical removal of protein from protein-capped DNA damage might be a prerequisite for subsequent initiation of DNA damage responses.

In addition to telomere-associated enzymes, many enzymes tend to remain on DNA [4-6] which might have a similar capping effect and prevent DNA damage being recognized. The best known example is DNA-enzyme-drug cleavable complex where topoisomerase is trapped covalently on DNA by topoisomerase-targeting drugs [4-6]. Several clinically useful antibacterial and anticancer drugs are known to promote the formation of topoisomerase-capped DNA breaks. It has been suggested that the efficacy of these drugs is greatly modulated by the pathways responded to this type of damage [3, 5, 6, 16]. Given the fact that protein-depleted telomere ends are recognized as DNA damage [8-13], the question arose as to how topoisomerase-capped DNA breaks can be processed to exposed DNA lesions for subsequent repair and checkpoint activation.

Through their ability to transiently break and rejoin DNA and subsequent conversion of topological isomers, DNA topoisomerases play essential roles in DNA metabolism, including transcription, replication and repair [5, 6, 17]. On the dark side, a protein-linked cleavable complex forms if the life time of reaction intermediate increased (e.g. blockage of religation step) [5, 6, 17]. Several characteristics distinguish the topoisomerase cleavable complex from other forms of DNA damage, most notably the reversibility and protein-linked DNA break [5, 6]. Because of the reversible nature of cleavable complexes, the lethality and other cellular responses depend on their irreversible processing to real DNA strand breaks [4-6]. In addition, the bulkiness of topoisomerase linked on DNA damage might prevent DNA breaks from damage detection in a manner similar to the protection of telomere ends by capping with telomere-associated proteins [4-6, 8-13]. Moreover, the cleavable complex also results in a discontinuity in the backbone of DNA and the blockage of DNA metabolisms [5, 6].

Substantial evidence indicate that helical tracking processes are critically involved in
processing topoisomerase-mediated DNA damage [4-6, 18-23], which might be important for
initiation of downstream events. Notably, blockage of DNA replication and RNA transcription
have been reported when cells are treated with topoisomerase drugs [4-6, 23, 24]. Active DNA
replication has been shown to convert cleavable complexes into real DNA breaks [4-6], which we
now refer this pathways as the replication-initiated processing (RIP) for topoisomerase cleavable
complex. Recent studies have pointed to the importance of RNA polymerases (RNAPs) and DNA
helicases in processing of topoisomerase covalent complexes [5, 21-23]. We, therefore,
concentrate our first year effect on dissecting the roles of TRIP and RIP pathways in initiation
downstream cellular responses followed the formation of topoisomerase cleavable complexes.

研究方法、結果與討論：

It has been established that drug-induced downregulation of topoisomerase is initiated by the
transcription collisions between RNAPs and cleavable complexes, followed by the proteolytic
degradation through the ubiquitin/26S proteasome system [5, 21-23]. Importantly, the fact that
tumor cells are generally defective in downregulation of the cleavable complexes, rendering their
hypersensitivity to camptothecin (CPT), a prototypic topoisomerase (TOP1) drug [22]. In the
present study, we designate this pathway as transcription-initiated processing (TRIP) of
topoisomerase-capped DNA damage. First, we demonstrated the activation of ATM kinase
activity upon treatments with any topoisomerase-targeting drugs. It was demonstrated by its
autophosphorylation, formation of ATM foci in CPT-treated cells and/or in vitro using an
immunoprecipitated ATM kinase assay.

First, we study the effect of TRIP and RIP on TOP1-capped DNA damage. CPT was used to
induce formation of TOP1-capped DNA damage. Using specific transcription and proteasome
inhibitors, we demonstrated that transcription collision of and/or the removal of TOP1 from
TOP1 cleavable complexes through TRIP pathway contribute to initiation of multiple
downstream DNA damage responses. In addition, DNA replication inhibitor was also employed
to investigate the effect of RIP on cellular responses induced by CPT. Significantly, these
TRIP-triggered DNA damage responses are distinct from the responses initiated through RIP
pathways of TOP1 cleavable complexes. Specifically, RPA phosphorylation was mainly initiated
through RIP pathway, while the elevated level of p53 could be triggered through both the RIP and
the TRIP pathways. In marked contrast, the phosphorylation of ATM kinase at Ser1981, p53 at
Ser15, Chk2 at Thr68 and Chk1 at Ser345 are more dependent on the TRIP pathway than the RIP
pathway. The TRIP pathway is unique for TOP1-linked DNA damage as evidenced by
irradiation-induced cellular responses were not affected by inhibition of transcription and
proteasome. The temporal order that TRIP of TOP1-capped DNA damage serves as ATM
upstream was further supported by blockage of CPT-induced ATM foci formation by transcription
and proteasome inhibitors. Taken together, these results support a TRIP-associated model in
which both collision between transcription and TOP1-capped DNA damage and subsequent
proteasomal degradation of TOP1 contribute to uncapping the TOP1-linked DNA strand breaks.
These processed DNA breaks can then be detected and sequentially trigger ATM, p53 and other
cellular responses.

It should be noted that TOP2-linked DNA damage is chemically different from TOP1-capped
DNA break in the following ways: (i) TOP2 causes double-strand breaks (DSBs), while TOP1
induces single-strand breaks (SSBs); (ii) The polarities of DNA covalently linked to enzyme are different, that is, TOP1 is linked to the 3’-end of cleaved DNA, whereas TOP2 is ligated to the 5’-terminus of DNA breaks. Therefore, we speculated that cellular responses followed processing pathways for TOP2-mediated DNA damage will be different from those of TOP-capped DNA damage. Toward this aim, we took similar approaches for TOP1-capped DNA damage mentioned above to study how TRIP and RIP affect the cellular responses initiated by TOP2-targeting drugs. Indeed, the effects of TRIP or RIP on cellular responses induced by TOP1- or TOP2-capped DNA breaks are distinct from each others. For example, phosphorylation of p53 at Ser15 was not affected by the TRIP pathway of TOP2-capped DNA damage, while it was nearly completely abolished by cotreatment of transcription inhibitor DRB with CPT. We also demonstrated in vitro that transcription did not convert TOP2 cleavable complex into irreversible TOP2-mediated breaks, which is different from transcription collision of TOP1 cleavable complex. Currently, we are investigating the effect of TRIP pathway on genomic instability induced topoisomerase drugs. We speculate that TRIP-mediated removal of enzymes from topoisomerase-capped DNA breaks is a prerequisite for topoisomerase-mediated recombinational mutations.

参考文献：
The cellular responses to protein-capped DNA damage remain largely unclear. In the present study, we used topoisomerase-DNA cleavable complex to study cellular responses to protein-capped DNA damage. The experiments reported here reveal that initiation of several topoisomerase-capped DNA damage-induced cellular responses specifically depends on the proteolytic removal of TOP1 from TOP1-capped DNA damage. In addition, two mechanistically distinct processing pathways for exposing topoisomerase-capped DNA damage have been identified, namely transcription- and replication-initiated processing (TRIP and RIP) of TOP1-mediated DNA breaks. As a result, blocking TRIP or RIP pathways of TOP1-linked DNA damage significantly modulates downstream signaling pathways activated by CPT. Therefore, tempering of the TRIP and/or the RIP pathways might present a novel intervention strategy for enhancing sensitivity of cancer cells and/or preventing clinical side effects exhibited by topoisomerase-targeting therapeutic drugs. Along this line, the topoisomerase drug-induced apoptosis and/or cell killing are expected to be complicated. Our preliminary results showed that APH had only a minimal effect on the formation of apoptotic ladders despite of its large preventive effect on cytotoxicity induced by CPT. On the other hands, MG132 reduced apoptotic laddering triggered by CPT. Clearly, further studies are needed for clarifying the relationships between processing pathways and cell death pathways for protein-capped DNA damage.