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News & views

Cancer

Signature of a DNA stress reliever

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Certain patterns of mutations occur frequently in cancer. The culprit behind one mutational signature is now shown to be a cellular enzyme with the mundane role of relieving stress in supercoiled DNA.

Cancers are fuelled by changes in DNA, known as driver mutations, that tip cells towards uncontrolled multiplication. The mutations vary from tumour to tumour and from person to person, but some common patterns are emerging from analyses of thousands of genomes from cancerous and normal human tissues[1]. These analyses are providing reference sets of mutational 'signatures' that can be used to unpick the molecular processes underlying the mutations[2]. Much progress has been made in understanding the events that underpin signatures involving the mutation of single DNA 'letters', or bases. But less is known about the causes of mutational footprints characterized by small insertions and deletions (indels) of DNA. Writing in *Nature*, Reijns *et al.*[3] describe their comprehensive computational and experimental investigation of the foundations of one such signature.

The pattern of mutations studied by Reijns and colleagues is 'indel signature 4' (ID4), reported in the Catalogue of Somatic Mutations in Cancer, or COSMIC, database[2]. The authors examine the overlap between the genome deletions characteristic of ID4 and those induced by the erroneous activity of a cellular enzyme, DNA topoisomerase 1 (TOP1), at specific genomic sites (those in which ribonucleotides, a non-standard type of DNA letter, become embedded). TOP1 is an evolutionarily conserved enzyme[4] that has the essential role of untwisting and relaxing DNA that has become supercoiled – something that happens often when DNA is either replicated or transcribed into messenger RNA. In the process of alleviating torsional stress, TOP1 can introduce deletions of between two and five base pairs, usually in short repeated sequences that Reijns *et al.* named short short tandem repeats[5].

Previous studies of yeast[5,6] have identified patterns of DNA deletions that are found only in cells with functional TOP1. In their work, Reijns and colleagues first show that the deletion patterns seen in yeast are similar to the ID4 signature in cancer. They then go a step further, developing a 'reporter' assay – based on fluorescent marker proteins – to detect mutational events that are generated by TOP1 activity in various organisms. The authors use this assay, together with whole-genome sequencing, to identify and validate the incidence of signatures similar to that of ID4 across other biological systems, including human cancer cell lines and mouse models of cancer. Their results provide comprehensive evidence that the mutagenic activity of TOP1 is conserved in different cellular contexts, different disease states and even different organisms.

Notably, Reijns and colleagues also show that TOP1 exhibits a striking sequence and transcriptional specificity in human cells. They find that the enzyme preferentially cleaves phosphodiester bonds in DNA that are adjacent to ribonucleotides with a thymine

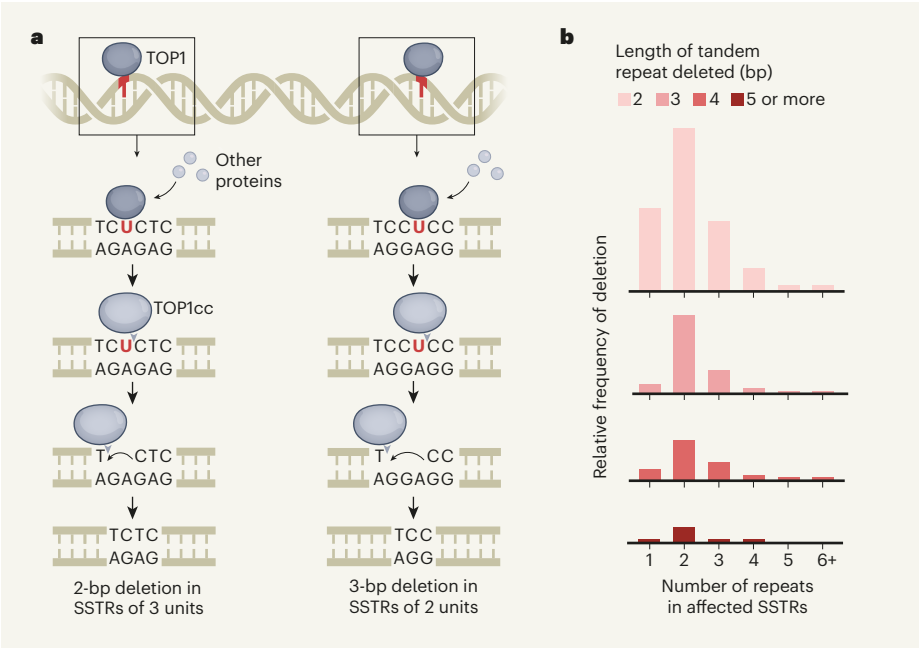

Figure 1 | Molecular foundations of a mutational footprint in cancer. Reijns *et al.*[3] propose a mechanism by which the enzyme DNA topoisomerase 1 (TOP1) induces patterns of DNA deletions across the genome. **a**, In this mechanism, TOP1 binds to DNA at atypical components called ribonucleotides (such as ribouridine (U), indicated in red) in DNA sequences known as short short tandem repeats (SSTRs). Here, two SSTRs that involve repeats of the DNA bases thymine (T) and cytosine (C) are shown, the first involving three repeated units of a two-base-pair (2-bp) sequence, the second two repeats of a 3-bp sequence; in each case, U has replaced T in one of the repeat sequences. Bound TOP1 recruits proteins to form the TOP1 cleavage complex (TOP1cc), which cleaves DNA at U. A second cleavage occurs, generally at an upstream T, leading to deletion of the repeat unit between the nicks. **b**, This error-prone excision of ribonucleotides generates genome-wide mutations that have a characteristic pattern, in which SSTRs – especially those in which a sequence is repeated one to three times – commonly carry deletions of repeats between 2 and 5 bp long. The authors find that this footprint of SSTR deletions resembles the ID4 mutational signature[2] seen in cancer. They also find it in eggs and sperm (perhaps thereby affecting evolution).

(T) base positioned upstream. More specifically, TOP1 induces deletions at sites that have two thymine bases positioned at least one base apart (known as TNT sites) in highly transcribed regions, both *in vitro* and *in vivo* (Fig. 1). Moreover, although TOP1 causes some deletions in non-transcribed regions, many more occur in transcribed regions. Together, all of this suggests that transcription-associated mutations induced by TOP1 largely drive the prevalence of the ID4 signature across the genome. The authors also identify this signature in the human germ line (the cells that form eggs and sperm), showing the same sequence and transcriptional specificity. The findings warrant further investigation into the role of TOP1 activity in both the development of cancer and human evolution.

The current results raise the prospect of using the ID4 footprint in the clinic. The signature could be used as a biomarker, providing clues to a cancer's susceptibility to TOP1 inhibitors, a mainstay of many treatment regimens[7]. However, first we must learn more about how the signature varies between cancer cells that do or do not have the working DNA-repair machinery required to fix TOP1's mistakes. Furthermore, a better mechanistic understanding of TOP1's mutational alter ego could pave the way to uncovering the roles and footprints of other classes of topoisomerase in human cancers.

Previous work[8] has shown that topoisomerase II beta (TOP2B) drives gene fusions in prostate cancer. Unfortunately, some individuals who are treated with a TOP2B inhibitor — which prevents the rejoining of broken DNA ends — develop therapy-related acute myeloid leukaemias, suggesting a need for normal TOP2B activity in preventing secondary tumours[9]. A study design similar to that of Reijns and colleagues might be used to understand the role of TOP2B and to identify its mutational footprint in primary and secondary cancers.

These findings also support the idea of using combination therapies to treat cancers that have DNA-repair deficiencies as well as TOP1 mutational activity. Cancer cells that have deficient DNA-repair pathways are vulnerable to types of DNA damage that can ultimately lead to genomic instability and cell death. These vulnerabilities might offer an opportunity to treat cancer by using TOP1 inhibitors together with drugs that prevent such DNA damage from being mended (such as PARP inhibitors or DNA-damage-response inhibitors, which are already being used individually for cancer treatment). Phase I clinical trials of combination therapies are already looking promising for treating some advanced malignancies[10,11], and merit further investigation for other cancers.

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