

On the origin of prostate fusion oncogenes

Jiri Bartek, Petra Hamerlik & Jiri Lukas

A new study reports that androgen signaling induces DNA double-strand breaks and *TMPRSS2-ERG* rearrangements through androgen receptor-mediated recruitment of topoisomerase 2B. These findings shed light on the generation of the most common fusion oncogene in human cancer.

Recent discoveries have established that the majority of prostate cancers harbor oncogenic gene fusions, thereby shifting the earlier paradigm that such chromosomal rearrangements are typical only for hematological tumors and sarcomas¹. The prostate cancer fusions commonly contain 5' genomic elements from androgen-regulated genes fused to members of the Ets family of transcription factors, resulting in aberrant androgen-driven oncogenic transcription^{1,2}. Despite their emerging pathogenetic and clinical importance², the mechanism through which such recurrent chromosomal rearrangements occur has been elusive. On page 668 of this issue, Srinivasan Yegnasubramanian and colleagues³ describe a key role for transcription-associated DNA breakage at the androgen receptor target genes involved in the fusion most commonly seen in prostate cancer (*TMPRSS2-ERG*). They show that such breaks are mediated by the DNA-processing enzyme topoisomerase 2B (TOP2B), which is recruited to these sites through physical association with the androgen receptor. These findings further our understanding of the molecular basis of prostate cancer development and have broader implications for cancer biology, genetics and oncology.

DNA breakage and transcription

Although DNA double-strand breaks (DSBs) form physiologically, for example, during maturation of antigenic receptor genes in lymphocytes or during DNA replication in

proliferating cells, the fidelity of such processes can be compromised through both environmental and endogenous factors, thereby destabilizing the genome and potentially leading to life-threatening disorders⁴. Indeed, aberrantly enhanced DSBs are a hallmark of cancer, and some DSBs give rise to cell type-specific fusion oncogenes through illegitimate repair^{5,6}. Because prostate cancer-associated fusions involve androgen receptor-regulated genes², and estrogen receptor-regulated transcription involves formation of local DSBs by TOP2B⁷, Haffner *et al.*³ hypothesized that androgen receptor signaling may lead to TOP2B-mediated DNA breakage and thereby fuel the formation of *TMPRSS2-ERG* fusions commonly seen in prostate cancer.

To test their hypothesis, the authors first knocked down or chemically inhibited TOP2B and showed that TOP2B is required for efficient androgen-mediated gene expression in human prostate cancer cells³. In a series of elegant genetic and imaging experiments, they further demonstrated that TOP2B is directly recruited by androgen receptors to promoter regions known to be involved in *TMPRSS2-ERG* fusions, that the ensuing local DSBs are attributable to TOP2B activity and that such DSBs are indeed recognized by DNA-damage signaling factors, including ATM kinase³. Finally, their results showed that such DSBs mediated by androgen receptor-TOP2B were highly recombinogenic, giving rise to *de novo* generation of *TMPRSS2-ERG* fusions, and that these chromosomal rearrangements could be prevented by inhibiting repair factors involved in sealing broken DNA ends³. A simplified model summarizing this mechanism is shown in **Figure 1**. The model also highlights major risk factors for prostate cancer, such as oxidative stress, chronic inflammation and dietary factors, which are all known to impair the

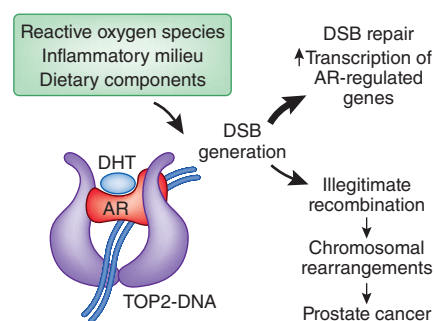


Figure 1 Model of androgen-stimulated generation of oncogenic chromosomal rearrangements. Androgenic signaling by dihydrotestosterone (DHT) induces recruitment of the AR-TOP2B complex to androgen receptor target genes, where TOP2B catalyzes DSBs to relieve DNA topological constraints during transcription³. Most such DSBs are correctly repaired, leading to legitimate gene expression. However, in rare cases, DSBs are fused with illegitimate partner genes to form oncogenic fusions such as *TMPRSS2-ERG*, a process promoted by reactive oxygen species (ROS), dietary constituents or inflammation, which are known risk factors for prostate cancer. AR, androgen receptor.

otherwise transient TOP2B-DNA complexes and thereby enhance the probability of local DSB formation and generation of fusion oncogenes (**Fig. 1** and refs. 2,3,8).

Implications and future challenges

These exciting new findings raise a host of challenging questions and advance our knowledge of prostate cancer pathogenesis, with potentially important implications for cancer treatment and prevention.

From a conceptual point of view, we face the puzzle of how prostate cancer cells or their precursor lesions—the prostatic intra-epithelial neoplasia—escape from permanent cell cycle arrest (cellular senescence) or avoid

Jiri Bartek, Petra Hamerlik and Jiri Lukas are at the Centre for Genotoxic Stress Research, Danish Cancer Society, Copenhagen, Denmark, and the Institute of Molecular and Translational Medicine, Palacky University, Olomouc, Czech Republic.
e-mail: jb@cancer.dk or jil@cancer.dk

undergoing cell death. Such cell fates are usually induced by activation of DNA-damage signaling^{4,5}, similar to that illustrated in this study in response to androgen-stimulated DSBs³. The replication stress- and DSB-inducible DNA damage response machinery emerges as an intrinsic barrier that prevents propagation of cells harboring activated oncogenes and blocks tumor progression in other major epithelial malignancies^{5,9,10}. To what extent this concept applies to prostate cancer—and whether the ability to rapidly seal the transient TOP2B-generated DSBs³ (as compared to persistent DSB signaling seen in cells undergoing oncogene-induced senescence^{5,11}) might explain the distinct impact on cell growth—remains to be elucidated.

Another key question is the extent to which the AR-TOP2B model of DSB and oncogenic-fusion formation is applicable to other transcription factors, many of which have oncogenic potential analogous to the androgen receptor. Is this mechanism largely restricted to nuclear hormone receptors such as the androgen receptor and the estrogen receptor, or do other transcription factors also recruit TOP2B⁷ to help resolve topological DNA entanglements formed during transcription? For example, TOP2-mediated DSBs can also form when replication forks encounter highly transcribed chromosomal regions¹². These and other scenarios require recombinogenic repair,

the malfunction of which could result in generation of rearrangements and fusion genes in other types of common epithelial tumors.

From a clinical standpoint, the new data suggest that care should be taken to consider potential risks of chemotherapy with TOP2B inhibitors such as etoposide or doxorubicin, which are known to enhance DSBs and cause drug-induced leukemogenic gene fusions¹³. Such treatments might also facilitate the androgen receptor-driven oncogenic fusions associated with prostate cancer. On a more positive note, the fact that the *TMPRSS2-ERG* fusions require the androgen receptor and can be prevented by inhibitors of repair enzymes PARP1 or DNA-PK³ suggests that modulation of androgen signaling or selected repair pathways might help prevent the generation of prostate cancer fusion oncogenes that are associated with aggressive disease².

Although assessing the overall impact of gene fusions in cancer may require powerful high-throughput technologies, the present identification of nonhomologous end-joining repair machinery as critical for generating *TMPRSS2-ERG* fusions helps us better understand why this particular event is apparently the most frequent genetic rearrangement identified to date. Whereas other oncogenic translocations occur between genes on different chromosomes that happen to be juxtaposed due to their positioning in defined nuclear territories¹⁴, *TMPRSS2*

and *ERG*^{3,15} are located only 3 Mb apart on chromosome 21. Given that proximity is a prerequisite for such translocations to occur, it might seem counterintuitive that evolutionary forces have not selected against the colocalization of these two genes *in cis*. However, it seems likely that this potentially hazardous gene juxtaposition is not associated with a substantial fitness cost because prostate cancer typically develops at an advanced age, thereby buffering this particular genomic configuration from influencing reproductive success.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Variation across the allele frequency spectrum

Anna L Gloyn & Mark I McCarthy

A new study finds that individuals with high plasma triglyceride levels carry approximately twice as many rare, coding genetic variants within four candidate genes identified through genome-wide association studies than individuals without these high levels. This study demonstrates the overlap of rare and common variant signals at loci associated with lipid levels and shows the value of efforts to extend susceptibility variant discovery to embrace the full allele-frequency spectrum.

Common-variant genome-wide association studies (GWAS) have, in recent years, been responsible for substantial advances in dissecting the genetic basis of common disease. However, for many of these traits, much of the genetic variation influencing individual risk of disease remains unexplained¹, and discovery efforts are now increasingly focused on

sources of genetic variation poorly captured by existing GWAS approaches. One clear target of these new approaches, powered by the availability of next-generation sequencing technologies, lies in enumerating the contribution to phenotypic variation made by low-frequency (minor allele frequencies in the 0.3–5.0% range) and rare (minor allele frequencies below 0.3%) genetic variants¹. On page 684 of this issue, Robert Hegele and colleagues combine these approaches by resequencing within associated loci from a GWAS for high triglyceride levels. By demonstrating that both common and low-frequency alleles at these loci are associated with

levels of circulating triglycerides, this study provides important clues to the architecture of variants influencing common biomedical traits².

Lipid genetics

Circulating levels of particular lipids represent important risk factors for coronary artery disease, and individuals with extreme values of these traits are often at high risk of myocardial infarction and early death. To date, GWAS have identified over 30 loci influencing lipid levels^{3,4}, including a dozen affecting triglycerides. These previous studies included individuals (recruited from population cohorts or ascertained for

Anna L. Gloyn and Mark I. McCarthy are at the Oxford Centre for Diabetes, Endocrinology and Metabolism, University of Oxford, Churchill Hospital, Oxford, UK.
e-mail: mark.mccarthy@drl.ox.ac.uk